colorizing carbon and filtered. The imidazole was precipitated from the filtrate by neutralizing with 6 *N* hydrochloric acid. It was then recrystallized from nitrobenzene following which the crystals were warmed with toluene and then petroleum ether and dried.

Preparation of 2-Substituted-1H-naphthimidazole-4,9-diones. Base-catalyzed Ring Closures. 2-Methyl-1H-naphthimidazole-4,9-dione.—To a hot solution of 4 g. of 2-acetamido-3-amino-1,4-naphthoquinone (0.017 mole) in 160 ml. of ethyl alcohol was added 20 ml. of 2 *N* sodium hydroxide. The mixture was heated for 15–20 minutes until the violet coloration changed to a brownish-orange. The solution was poured into 600 ml. of water containing 20 ml. of 2 *N* hydrochloric acid. The mixture was heated and alcohol gradually added until the imidazole, which had separated, redissolved. The hot solution was treated with decolorizing carbon, filtered and cooled. The product separated as yellow needles and was recrystallized from ethyl alcohol. All of the imidazoles in this series were yellow, crystalline compounds.

2-Ethyl-1H-naphthimidazole-4,9-dione.—A solution of 12 g. (0.049 mole) of 2-propionamido-3-amino-1,4-naphthoquinone in 480 ml. of alcohol and 60 ml. of 2 *N* sodium hydroxide was distilled *in vacuo* to 200 ml. and the solution was acidified with 6 *N* hydrochloric acid. The product was dissolved in warm dilute sodium hydroxide and reprecipitated, after treating with decolorizing carbon, with hydrochloric acid. The product was recrystallized from ethyl alcohol.

Preparation of 2-Substituted-1H-naphthimidazole-4,9-diones. Acid-catalyzed Ring Closure. 2-Methyl-1H-naphthimidazole-4,9-dione. (a).—Two grams of 2-acetamido-3-amino-1,4-naphthoquinone in 50 ml. of glacial acetic acid was boiled with an excess of zinc turnings until the red color changed to yellow (about 10 minutes). Additional acetic acid was added to dissolve the blue precipitate which had formed. The hot solution was treated with decolorizing carbon, filtered and poured into four volumes of water whereupon yellow needles separated. More product was obtained from the filtrate by adjusting to a *p*H of 8, bubbling air through the solution until the color became orange and acidifying, total yield 65%. It was recrystallized from ethyl alcohol, m.p. 368°.

(b).—Hydrogen was passed into a boiling solution of 2 g. of 2-acetamido-3-amino-1,4-naphthoquinone in 50 ml. of glacial acetic acid, in the presence of 100 mg. of palladium black, until the solution became dark yellow. The palladium was removed and the acetic acid removed under reduced pressure. The residue was suspended in 200 ml. of water and allowed to stand with occasional shaking for 24 hours, or until the suspended solid became yellow. Sodium hydroxide (12 *N*) was added to dissolve the imidazole. The solution was treated with decolorizing carbon and acidified, yield 71%. The product was recrystallized from ethyl alcohol, m.p. 368°.

2-(N-Diethylaminomethyl)-1H-naphthimidazole-4,9-dione.—A boiling solution of 15.07 g. (0.05 mole) of 2-diethylaminoacetamido-3-amino-1,4-naphthoquinone in 100 ml. of glacial acetic acid, in the presence of 300 mg. of palladium black was treated with hydrogen as above. After removing the acetic acid *in vacuo*, the residue was dissolved in excess of warm 2 *N* sodium hydroxide and diluted to 500 ml. Oxygen was passed into the solution for 15 minutes. The solution was decolorized, acidified with hydrochloric acid and cooled, yield of hydrochloride 76%. The product was recrystallized from water and dried at 120° *in vacuo*.

The hydrochloride was converted to the free base by passing a solution in 50% alcohol through a column of anion exchange resin, IR4B(OH). The resulting solution, which was free of chloride ion, was distilled *in vacuo* and the yellow residue recrystallized from toluene, yield 72%.

2-Piperidinomethyl-1H-naphthimidazole-4,9-dione.—The above procedure was applied to 2-piperidinoacetamido-3-amino-1,4-naphthoquinone.

2-Morpholinomethyl-1H-naphthimidazole-4,9-dione.—2-Morpholinoacetamido-3-amino-1,4-naphthoquinone was subjected to the above procedure.

PHILADELPHIA 4, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Researches on Substituted 5-Phenylhydantoins[1]

BY HENRY R. HENZE AND ARTHUR FURMAN ISBELL[2]

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Twelve new 5-(substituted phenyl)- or 5,5-di-(substituted phenyl)-hydantoins have been synthesized. Catalytic hydrogenation of 5-(4-methylphenyl)-5-phenylhydantoin attacks only the unsubstituted phenyl grouping.

While numerous 5-phenyl-5-substituted hydantoins have been tested for pharmacological activity,[3] very little has been learned concerning the activities of 5-(substituted phenyl)-hydantoins.[4] It was of interest, therefore, to prepare other substituted-phenyl hydantoins so that their anticonvulsant activities, if any, might be compared with that of the potent 5,5-diphenylhydantoin or 5-ethyl-5-phenylhydantoin.

The first group of hydantoins, synthesis of which is reported in this paper, is monosubstituted derivatives of 5,5-diphenylhydantoin and were obtained through utilization of appropriate ketones, namely: $C_6H_5COC_6H_4$–X(*para*) where X = amino, hydroxy or methoxy. Another group illustrated disubstituted types derived from (*p*)-X–$C_6H_4COC_6H_4$–X-(*p'*), where X = amino or chloro. The third class, related to 5-ethyl-5-phenylhydantoin, was prepared from ketones of the type $C_2H_5COC_6H_4$–X, where X = bromo, hydroxy or methoxy. Of the various individual derivatives prepared, that produced from 4,4'-diaminobenzophenone, namely, 5,5-di-(4-aminophenyl)-hydantoin, possessed added interest. The parent ketone had been shown to possess a sulfonamide-like action in that it inhibits the growth of bacteria, this inhibition being overcome by *p*-aminobenzoic acid.[5] Obviously, testing of this hydantoin derivative would be desirable in order to ascertain whether this inhibition was retained in the hydantoin.

The replacement of one or both of the phenyl groups in 5,5-diphenylhydantoin with an equal number of cyclohexyl groups apparently causes

(1) From the Ph.D. dissertation of A. F. Isbell, June, 1943.

(2) University Advanced Research Fellow, The University of Texas, 1942–1943.

(3) H. H. Merritt and T. J. Putnam, *Epilepsia*, **3**, 51 (1945). Although many 5-phenyl-5-substituted hydantoins have been shown to possess some degree of anticonvulsant activity, an increasing number of such compounds is being found to be devoid of such activity. Moreover, several 5,5-disubstituted hydantoins having no phenyl substituent are known to be active anticonvulsants.

(4) J. W. Melton and H. R. Henze, THIS JOURNAL, **69**, 2018 (1947), have reported on the preparation of 5-(*meta*-substituted phenyl)-hydantoins; without exception, these compounds have been found to lack anticonvulsant activity.

(5) R. Kuhn, E. F. Moller, G. Wendt and H. Binert, *Ber.*, **75B**, 711 (1942).

little loss of anticonvulsant activity. For this reason hydantoin derivatives have been prepared in order to permit determination of the effect of introduction of an alkyl radical into the phenyl- or cyclohexylhydantoins through combination of pairs of groups such as phenyl and 4-methylphenyl, cyclohexyl and 4-methylphenyl, phenyl and 4-methylcyclohexyl, and cyclohexyl and 4-methylcyclohexyl.

From time to time only partially successful attempts have been made to convert drugs of pronounced pharmacological activity into colored compounds in which all or a portion of such activity was retained. For example, colored derivatives of nirvanol[6] have been synthesized.[7]

Since 5-(4-aminophenyl)-5-phenylhydantoin has been found to possess definite anticonvulsant activity, it was decided to attempt to prepare azo derivatives of 5,5-diphenylhydantoin through diazotization of the amino derivative with subsequent coupling with suitable aryl amines or phenols.

In this investigation there have been prepared twelve new 5-monosubstituted or 5,5-disubstituted hydantoins in which at least one of the substituents is a substituted-phenyl group and the other group is either another substituted-phenyl, or alkyl or hydrogen. In the case of the monosubstituted hydantoins or those containing an alkyl group at the 5-C position, synthesis was accomplished by condensation of the appropriate ketone, potassium cyanide and ammonium carbonate in diluted alcohol. However, when the starting ketone was a substituted benzophenone, the limited solubility of the latter in diluted alcohol and the slow rate of hydantoin formation necessitated the use of the acetamide method devised in this Laboratory.[8]

An attempt was made to reduce 5-(4-methylphenyl)-5-phenylhydantoin through hydrogenation in the presence of the Adams platinum catalyst and hydrochloric acid.[9] Although hydrogen was utilized fairly rapidly, in no instance was the consumption of hydrogen in excess of the amount theoretically required for the reduction of only one phenyl ring. Likewise, analysis of the reaction product established that partial reduction to a hexahydro derivative had occurred.

In order to determine in this instance which of the two benzene rings had been reduced, it was necessary to synthesize two isomeric ketones, namely, cyclohexyl 4-methylphenyl ketone and 4-methylcyclohexyl phenyl ketone, and to convert each into the corresponding hydantoin. Comparison of these hydantoins with the product from the catalytic reduction showed that the compound in question is 5-cyclohexyl-5-(4-methylphenyl)-hydantoin. Other attempts to cause reduction of the substituted phenyl ring in this hydantoin were unsuccessful. Likewise, reduction of 5-(4-methylphenyl)-5-phenylhydantoin with Raney nickel catalyst under conditions of high temperature and pressure also resulted in hydrogenation only of the phenyl group. Catalytic reduction of 5-(4-methylcyclohexyl)-5-phenylhydantoin yielded the anticipated 5-cyclohexyl-5-(4-methylcyclohexyl)-hydantoin. The latter was synthesized also from interaction of cyclohexyl 4-methylcyclohexyl ketone with potassium cyanide and ammonium carbonate in fused acetamide solution.

Considerable difficulty was encountered in the conversion of 4,4′-diaminobenzophenone into 5,5-di-(4-aminophenyl)-hydantoin, although 5-(4-aminophenyl)-5-phenylhydantoin was synthesized from 4-aminobenzophenone in a yield of 62.5%. In order to obtain the diaminophenyl derivative it was found necessary to prepare the N,N′-diacetyl derivative of 4,4-diaminobenzophenone, and to convert it into the corresponding hydantoin which, subsequently, was hydrolyzed to remove the acetyl groups.

Through the courtesy of Parke, Davis and Company, seven of the substituted hydantoins prepared in this investigation received preliminary screening for possible anticonvulsant activity. Only 5-(4-aminophenyl)-5-phenylhydantoin exhibited any activity, and it only perhaps one-half that of 5,5-diphenylhydantoin. However, this amino derivative is capable of forming a stable hydrochloride salt and the latter is far more water-soluble than is the parent compound. Apparently, substitution on the 4-position of the phenyl group reduces or entirely eliminates the anticonvulsant activity of 5,5-diphenylhydantoin.

Experimental

Preparation of 5,5-Disubstituted Hydantoins.—The preparation of these hydantoins was attempted first employing the conditions developed by Bucherer and Lieb,[10] namely, use of diluted alcohol as the solvent and 55–60° as the temperature for reaction. In general, these conditions proved unsatisfactory and in the cases of 4,4′-dichlorobenzophenone, 2,4′-dichlorobenzophenone, 4-aminobenzophenone and 4-hydroxybenzophenone, the ketone was essentially quantitatively recovered unchanged. By placing the alcoholic solution (or suspension) in a closed pressure vessel at 105–110°, it was possible to obtain adequate conversion of 4-hydroxypropiophenone and 4-methoxyacetophenone into the corresponding hydantoins, but for the remainder of the ketones, successful conversion required utilization of the procedure of Henze and Long[8] which involves use of fused acetamide as solvent with heating at 110°.

In general, one part (0.1 mole) of ketone was added to about 250 g. of fused acetamide, placed in the Pyrex liner of a pressure vessel, and mixed thoroughly with 1.1 parts of potassium cyanide (as such or dissolved in an equal weight of water). Three parts of ammonium carbonate was introduced and the liner placed in the container and heated in an oven at 110° for from 9 to 48 hours. After cooling, the container was opened and the contents treated with about 500 ml. of boiling water. Insoluble material, if present, was removed by filtration and the filtrate was acidified to obtain additional product. Unreacted ketone could be recovered by washing the crude product repeatedly with small amounts of ether (in which the hydantoins had but limited solubility), or by treating the reaction product with cold 10% alkali solution and filtering or removing the ketone by ether extraction. The hydantoin was obtained by acidification with concentrated hydrochloric acid, except where amino groups were present. For further purification,

(6) J. J. Spurlock with H. R. Henze, THIS JOURNAL, **60**, 3005 (1938); S. P. Lingo with H. R. Henze, *ibid.*, **61**, 2029 (1939).

(7) Certain of these derivatives proved to be quite painful when injected into animals; hence, these compounds have not received additional testing and no experiments were carried to autopsy. Therefore, no information as to the possible pharmacological activity of these azo compounds has been secured.

(8) H. R. Henze and L. M. Long, THIS JOURNAL, **63**, 1941 (1941).

(9) J. H. Brown, H. W. Durand and C. S. Marvel, *ibid.*, **58**, 1594 (1936), reduced the phenyl group to the cyclohexyl group with this catalyst activated by the presence of hydrochloric acid.

(10) H. Th. Bucherer and V. A. Lieb, *J. prakt. Chem.*, [2] **141**, 5 (1934).

TABLE I

5-R-5-R′-HYDANTOINS

R	R′	Yield, %	M.p., °C. (cor.)	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Halogen, % Calcd.	Halogen, % Found
4-ClC_6H_4-	4-ClC_6H_4-	94	319–320[a]					8.72	8.67	22.08	22.06[c]
4-ClC_6H_4-	2-ClC_6H_4-	88	292.0–292.5[b]					8.72	8.60	22.08	21.94[c]
4-$NH_2C_6H_4$-	C_6H_5-	81	295[b]	67.40	67.29	4.90	4.93	15.72	15.67		
4-HOC_6H_4-	C_6H_5-	68	306[b]	67.18	67.09	4.51	4.44	10.45	10.23		
4-$CH_3OC_6H_4$-	C_6H_5-	76	227–228	68.07	67.93	5.00	4.95	9.93	10.11		
4-HOC_6H_4-	C_2H_5-	89	268.0–268.5[b]	59.99	59.89	5.49	5.40	12.72	12.70		
4-$CH_3OC_6H_4$-	CH_3	84	210–212	59.99	59.81	5.49	5.41	12.72	12.84		
4-$CH_3C_6H_4$-	C_6H_5	87	229–230	72.16	72.07	5.30	5.39	10.52	10.45		
4-BrC_6H_4-	CH_3	74	276.5[b]					10.41	10.33	29.70	29.64[d]
4-$(C_2H_5)_2NC_6H_4$-	H	81	177.5–178.5	63.14	63.16	6.93	6.73	16.99	17.23		
4-$NH_2C_6H_4$-	4-$NH_2C_6H_4$-	38	311[b]	63.82	64.07	5.00	5.07	19.85	19.29		
4-$CH_3C_6H_4$-	4-$CH_3C_6H_4$-	82	237–238	72.84	73.01	5.75	5.85	10.00	10.14		
4-$CH_3C_6H_4$-	C_6H_{11}-	98	252.5–253.5	70.56	70.45	7.40	7.30	10.26	10.14		
4-$CH_3C_6H_{10}$-	C_6H_5-	86	243–244	70.56	70.46	7.40	7.89	10.29	10.09		
4-$CH_3C_6H_{10}$-	C_6H_{11}-	87	275–276	69.03	68.87	9.41	9.45	10.06	10.22		
4-$CH_3C_6H_{10}$-	4-$CH_3C_6H_{10}$-	99	297–298[b]	69.82	69.99	9.65	9.76	9.58	9.70		

[a] Uncorrected m.p. [b] M.p. with decomposition. [c] Analysis for chlorine. [d] Analysis for bromine.

glacial acetic acid or ethyl alcohol had some value, but dioxane proved to be the best solvent for these phenylhydantoins. Data concerning these hydantoins may be found in Table I.

Purification of 5-(4-aminophenyl)-5-phenylhydantoin proved troublesome until a few crystals of hydroquinone were added to the solvent. Quite a deal of difficulty was encountered in the preparation of the hydantoin from 4-diethylaminobenzaldehyde. The first attempt was made with dilute alcohol as solvent, the mixture being heated at 55–60° for ten hours. After adjusting the *p*H to 6, and removal of solvent, the reaction product consisted of a very dark, oily liquid and a small amount of faintly pink, amorphous solid which darkened at about 270° and decomposed at 305°. The solid was essentially insoluble in most of the common organic solvents and in dilute alkali, although soluble in dilute acid. Repetition of the attempt with warming for 24 hours gave the same results.

Since McKee[11] had been successful in converting the semicarbazone of a ketone directly into the hydantoin derivative, the semicarbazone of 4-diethylaminobenzaldehyde (m.p. 216–217° (cor.) dec.[12] was prepared and heated with potassium cyanide and ammonium carbonate in fused acetamide at 110° for 16 hours; only a very small amount of the material decomposing at 305° resulted.

Following the usual procedure,[13] 4-diethylaminobenzaldehyde cyanohydrin was prepared and dissolved in ethyl alcohol together with potassium cyanide and ammonium carbonate and heated at 55–60° for 10 hours. After removal of the alcohol, the residue was made strongly acidic, the solution was filtered and brought to a *p*H of 6 by addition of sodium hydroxide solution. After filtration from some gummy material, Norit was added; after warming, filtering and addition of glacial acetic acid, more gummy material separated, but the latter became granular when the mixture was boiled. The solid was separated and dried in a vacuum desiccator; its weight represented a yield of 81%. Solution of the product in dioxane–water produced a deep red solution from which a small amount of white, flocculent solid separated slowly during a period of three days. Solutions of the solid in hot alcohol–water or hot acetone–water were less colored but gave the same result. When a solution in diluted ethyl alcohol was boiled for 20 minutes, an unidentified product of melting point 305° dec. was produced. Chilling alcohol solutions of the initial product caused separation of a light tan, crystalline material, m.p. 171–173° dec. This product was both acid and alkali soluble, and was very soluble in methanol, even at 4°. By chilling such a solution, there were obtained crystals with a faint pink tint, m.p. 177.5–178.5° dec.

Anal. Calcd. for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99. Found: C, 63.16; H, 6.73; N, 17.23.

Preparation of 5,5-Di-(4-aminophenyl)-hydantoin.—Fifteen and nine-tenths grams (0.075 mole) of 4,4′-diaminobenzophenone, 5.37 g. (0.0825 mole) of potassium cyanide and 25.7 g. (0.225 mole) of ammonium carbonate in 250 g. of acetamide were heated together at 110° for nine hours. No alkali-soluble material was obtained and 11.4 g. of the ketone was recovered.

The reaction was attempted again using half the amounts of reagents noted above, except with substitution of 150 ml. of propylene glycol for the acetamide as solvent. The mixture was heated at 140° for 23 hours; 5.75 g. of ketone was recovered and no alkali-soluble product was found.

A sample of 4,4′-dibenzalaminobenzophenone (m.p. 200° (cor.) was prepared according to the directions of Fierz and Koechlin.[14] This material was used with 50 ml. of propylene glycol as the solvent in an attempt to obtain the desired hydantoin derivative; after heating at 110° for 20 hours, about 1 g. of the original amino ketone was recovered, the residual organic material being a non-alkali-soluble, gummy tar.

The dibenzoyl derivative (m.p. 230–231°) of 4,4′-diaminobenzophenone was prepared and heated in acetamide solution with cyanide and ammonium carbonate at 110° for 31 hours in an unsuccessful attempt to bring about conversion into a hydantoin.

However, heating the diacetyl derivative (239–240°) of this amino ketone in acetamide with ammonium carbonate and a saturated aqueous solution of potassium cyanide in a closed container at 110° for four days did lead to the hydantoin. The reaction mixture was heated with concentrated hydrochloric acid to remove acetic acid, diluted with water causing precipitation of solid material. The latter was extracted with 10% sodium hydroxide solution, leaving most of the unreacted amino ketone; neutralization with glacial acetic acid produced the hydantoin derivative (38% yield).

Reduction of 5-(4-Methylphenyl)-5-phenylhydantoin.—Ten grams of this derivative was partially dissolved in 170 ml. of warm ethyl alcohol and treated with 12.5 ml. of concentrated hydrochloric acid and 0.4 g. of Adams platinum catalyst. This mixture was shaken with hydrogen, under about 1.5 atmospheres pressure, for 36 hours. Then, another 0.4-g. portion of catalyst was added and the vigorous shaking under hydrogen was continued for another period of 36 hours. Practically all of the hydrogen utilized was absorbed during the first exposure to hydrogen. The mixture was heated to boiling, filtered from the catalyst, and concentrated to 100 ml. before water was added and the mixture chilled to cause separation of fine white needles, m.p. 241.5–242.5°. The melting point of a mixture of this material with 5-(4-methylphenyl)-5-phenylhydantoin (m.p. 229-

(11) R. L. McKee, Unpublished results from this Laboratory.

(12) F. Sachs and L. Sachs (*Ber.*, **38**, 525 (1905)) reported m.p. 214° dec.

(13) J. Houben, "Die Methoden der organischen Chemie," Georg Thieme, Leipzig, 3rd Ed., Vol. 2, 1925, p. 583.

(14) H. E. Fierz and H. Koechlin, *Helv. Chim. Acta*, **1**, 218(1918), reported m.p. 194° for the dibenzal derivative and 237° for the N,N′-diacetyl compound.

230°) was 215–220°. After exhaustive purification, this hydantoin melted at 252.5–253.5°. Analytical data indicated that only partial reduction had been accomplished to yield the hexahydro product, $C_{16}H_{20}N_2O_3$.

Anal. Calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40. Calcd. for $C_{16}H_{26}N_2O_2$: C, 69.03; H, 9.41. Found: C, 70.31; H, 7.62.

Where hydrogenation was carried out in very dilute solution, the product was obtained directly from the reaction mixture in a state of analytical purity.

In proof of the structure, cyclohexanecarboxylic acid,[15] b.p. 233° (746 mm.), was prepared, converted into its acid chloride,[16] b.p. 173–174° (750 mm.), which was condensed with toluene in the presence of anhydrous aluminum chloride to yield **cyclohexyl 4-methylphenyl ketone**, m.p. 66.5–67.5°.

Anal. Calcd. for $C_{14}H_{18}O$: C, 83.12; H, 8.97. Found: C, 83.11; H, 9.10.

An oxime was prepared and recrystallized from petroleum ether, as long, white needles; m.p. 169–170°.

Anal. Calcd. for $C_{14}H_{19}NO$: N, 6.45. Found: N, 6.55.

The structure of this ketone was demonstrated through its oxidation, with potassium permanganate in potassium hydroxide solution, to produce the known terephthalic acid.

Cyclohexyl 4-methylphenyl ketone (20.05 g., 0.099 mole), potassium cyanide (7.1 g., 0.109 mole), ammonium carbonate (34 g.) and 250 g. of molten acetamide were heated together at 110° for 11 hours. After one recrystallization from diluted dioxane, there was obtained 5-methylphenyl-5-cyclohexylhydantoin, m.p. 252.5–253.5°, 26.5 g. (98% yield), as fine white needles.

Anal. Calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.26. Found: C, 70.25; H, 7.30; N, 10.14.

The melting point of a mixture of this compound with the product from the reduction of 5-(4-methylphenyl)-5-phenylhydantoin was 252.5–253.5°. Therefore, in this reduction, the phenyl group was hydrogenated while the 4-methylphenyl group was unaffected.

Preparation of 5-(4-Methylcyclohexyl)-5-phenylhydantoin.—For this synthesis, 4-methylcyclohexanol was treated with phosphorus tribromide to produce 1-bromo-4-methylcyclohexane,[17] the latter converted into a Grignard reagent, and the latter treated with a toluene solution of benzonitrile. Thus was obtained **4-methylcyclohexyl phenyl ketone**, b.p. 151.0–152.5° (9 mm.), m.p. about 20°, d^{20}_4 1.0089, d^{40}_4 0.9959, $n^{20}D$ 1.5323, $n^{40}D$ 1.5238, ΣMR 61.70; MR found (20°) 62.15, (40°) 62.13.

Anal. Calcd. for $C_{14}H_{18}O$: C, 83.12; H, 8.97. Found: C, 83.01; H, 8.92.

An oxime formed readily from this ketone, but was rather difficult to purify; m.p. 145–146° (needle-clusters).

Anal. Calcd. for $C_{14}H_{19}NO$: N, 6.45. Found: N, 6.43.

Thirty-five grams (0.173 mole) of this phenyl ketone, 12.4 g. (0.19 mole) of potassium cyanide, 59 g. of ammonium carbonate and 250 g. of acetamide were heated at 110° for 14 hours. The alkali-soluble product was recrystallized from diluted alcohol; 40.6 g. of tiny needle crystals, m.p. 175–215°. Fractional crystallization of this material from glacial acetic acid produced various crops melting from 180–190° and 235–237°. All of this material except the highest melting fraction was combined and recrystallized from carbon tetrachloride; product, m.p. 227–228°. With the idea that this solid might contain some substituted hydantoic acid (corresponding to the desired hydantoin derivative), it was refluxed for two hours with concentrated hydrochloric acid; the m.p. behavior of the product was unchanged by this treatment. Recrystallization from diluted methyl alcohol produced clumps of fine white needles melting at 232–237°.

Anal. Calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Calcd. for $C_{16}H_{20}N_2O_2 \cdot H_2O$: C, 66.20; H, 7.64; N, 9.65. Found: C, 66.74; H, 7.81; N, 9.87.

A small sample of this hydrated material was sublimed, under 1 mm. pressure, to yield 5-(4-methylcyclohexyl)-5-phenylhydantoin (m.p. 242°), which was recrystallized from isoamyl alcohol–petroleum ether; m.p. 243–244°.

Anal. Calcd. for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.46; H, 7.89; N, 10.09.

Hydrogenation of 5-(4-Methylcyclohexyl)-5-phenylhydantoin.—This compound (7.0 g., 0.0257 mole) was dissolved in 150 ml. of ethyl alcohol in which 0.5 g. of Adams platinum catalyst was suspended; 10 ml. of concentrated hydrochloric acid was added and the mixture shaken under hydrogen. During 36 hours 0.77 mole (3 equivalents) of hydrogen was taken up, and much of the product had crystallized from solution; m.p. 275–276°. The 5-cyclohexyl-5-(4-methylcyclohexyl)-hydantoin, after recrystallization from diluted dioxane, melted at 275.5–276.5°. A mixture of this material and the hydantoin formed from cyclohexyl 4-methylcyclohexyl ketone melted unchanged.

Preparation of 4-Methylcyclohexyl 4-Methylphenyl Ketone.—A Grignard reagent was prepared from 176 g. (1 mole) of 4-methylcyclohexyl bromide in 1500 ml. of anhydrous ether and treated with an ether solution of 58.5 g. (0.5 mole) of *p*-tolunitrile. After refluxing the mixture for three hours, it was cooled and acidified; the ether layer was removed, dried and fractionated. Thus was obtained 49.4 g. (46% yield) of the desired ketone which boiled at 172–175° (6 mm.) and solidified in the receiver. After one crystallization from diluted alcohol it melted at 83–84°.

Anal. Calcd. for $C_{15}H_{20}O$: C, 83.28; H, 9.32. Found: C, 83.19; H, 9.25.

An oxime was produced in the form of long, thin, needle-like crystals which were recrystallized from diluted alcohol to melt at 176.5–177.5°.

Anal. Calcd. for $C_{15}H_{21}NO$: N, 6.06. Found: N, 5.95.

Preparation of 5-(4-Methylcyclohexyl)-5-(4-methylphenyl)-hydantoin.—In the usual manner, 22.0 g. (0.102 mole) of 4-methylcyclohexyl 4-methylphenyl ketone, 7.35 g. (0.113 mole) of potassium cyanide and 34.9 g. (0.306 mole) of ammonium carbonate were heated at 110° for 24 hours in molten acetamide. There was obtained 16.2 g. (55% yield) of the anticipated hydantoin. After recrystallization from dioxane–water and from glacial acetic acid, this hydantoin derivative melted at 234–235°.

Anal. Calcd. for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.15; H, 7.77; N, 9.90.

Hydrogenation of 5-(4-Methylcyclohexyl)-5-(4-methylphenyl)-hydantoin.—Three grams of this derivative was dissolved in 150 ml. of ethyl alcohol; 3 ml. of concentrated hydrochloric acid and 0.3 g. of Adams platinum catalyst were added and the compound was hydrogenated under an initial hydrogen pressure of about 1.5 atmospheres. After six hours, three equivalents of hydrogen had been used; thereafter, no additional gas was taken up. The initial product of reduction melted at 292–293°, but after recrystallization this sample of 5,5-di-(4-methylcyclohexyl)-hydantoin melted at 297–298°. This melting point was unchanged when this material was mixed with some of the product of hydrogenation of 5,5-di-(4-methylphenyl)-hydantoin.

Attempted Hydrogenation of 5-Cyclohexyl-5-(4-methylphenyl)-hydantoin. A.—This hydrogenation was attempted in ethyl alcohol, using the Adams platinum catalyst in the presence of hydrochloric acid. After exposure for 36 hours to hydrogen under pressure, no absorption of hydrogen had occurred and the unaltered hydantoin derivative was recovered.

B.—In another attempt, this hydantoin derivative (0.0075 mole) was treated with 5% hydriodic acid (5 ml.) and just enough glacial acetic acid to make a homogeneous solution. The latter was heated to reflux and 5-ml. portions of the hydriodic acid were added at the close of each five-hourly interval. Most of the compound recrystallized unchanged when the solution was chilled.

C.—The hydrogenation was reattempted with addition of red phosphorus to the hydriodic acid–acetic acid solution; the mixture was heated for 24 hours. Again, essentially quantitative recovery of unchanged 5-cyclohexyl-5-(4-methylphenyl)-hydantoin was obtained.

Hydrogenation of 5,5-Di-(4-methylphenyl)-hydantoin.—To 6 g. of 5,5-di-(4-methylphenyl)-hydantoin (m.p. 237–238°) dissolved in 300 ml. of ethyl alcohol were added 0.6 g. of the Adams platinum catalyst and 6 ml. of concentrated

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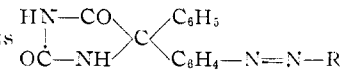
TABLE II

5-PHENYL-5-(*p*-SUBSTITUTED PHENYL-AZOPHENYL)-HYDANTOINS



	R	M.p., °C.	Yield, %	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found	Nitrogen, % Calcd.	Found	Color
1	4-HO-C_6H_4	277–278 d.	77	67.73	67.59	4.33	4.38	15.05	14.88	Bright yell.
2	4-HO-$C_{10}H_6$	278.5 d.	71	71.08	71.01	4.30	4.17	13.26	13.07	Dark red
3	2-HO-$C_{10}H_6$	294.5 d.	87	71.08	70.96	4.30	4.28	13.26	13.16	Vermillion
4	$(CH_3)_2N$-C_6H_4	274.0–274.5 d.	78	69.15	69.08	5.30	5.29	17.53	17.59	Orange
5	2-NH_2-6-SO_3H-$C_{10}H_5$	[a]	71	59.87	59.61	3.82	3.88	13.97	13.76	Maroon

[a] When heated, this material began to darken about 290°, but had not melted at 360°; on a platinum foil, heating caused the compound to swell to about ten times its original volume and to evolve much gas before burning slowly.

hydrochloric acid. The mixture was shaken at room temperature under hydrogen at a pressure of about 1.5 atmospheres. After 13 hours, the consumption of hydrogen ceased; there had been utilized about twelve mole-equivalents of hydrogen, and white, crystalline material had separated from the reaction mixture. Although a fresh portion of catalyst was added and shaking under hydrogen pressure was continued for two hours, no more gas was utilized. From the suspended solid was obtained 4.1 g. of small needle-like crystals of melting point 294–295° dec.; the filtrate gave an additional 1.7 g. of product m.p. 288–290° dec.; total yield 6.16 g. (98%). After repeated recrystallization from alcohol, 5,5-di-(4-methylcyclohexyl)-hydantoin melted at 297–298° dec.

Anal. Calcd. for $C_{17}H_{28}N_2O_2$: C, 69.82; H, 9.65; N, 9.58. Found: C, 69.99; H, 9.76; N, 9.70.

Preparation of 5-(Amino- or Hydroxyaryl-)-*p*-azophenyl-5-phenylhydantoin.—Usually, 10 g. (0.0375 mole) of 5-(4-aminophenyl)-5-phenylhydantoin was added to 6.5 ml. (0.075 mole) of concentrated hydrochloric acid and the mixture was stirred until a gummy mass resulted; the latter could be dissolved by addition of 100 ml. of water (this hydantoin does not dissolve readily in dilute acid). Chipped ice was added until the temperature of the solution reached 0°, 2.85 g. (0.0413 mole) of sodium nitrite (dissolved in 10 ml. of water) was added beneath the surface of the solution by means of a pipet. While the cold, diazotized mixture[18] was being stirred for 30 minutes, a solution was made of the compound to be coupled. In those cases where the coupling agent was an amine, 2.25 g. (0.0375 mole) of urea was added to the suspension of the diazonium compound and the stirring was continued for 15 minutes (to destroy excess nitrous acid) before the alcoholic solution of the amine (0.0375 mole) was added. If the compound to be coupled was acidic, 0.0375 mole of such material was dissolved in 500 ml. of water containing 3.0 g. (0.075 mole) of sodium hydroxide. To the well-stirred appropriate alcoholic solution (of an amine), or alkaline solution (of a phenol or sulfonic acid), chilled to 0°, was added the solution of the diazotized aminophenylhydantoin. Stirring was continued for 30 minutes after the addition had been made.

When the resulting azo compound did not contain a basic group, the product was readily caused to precipitate from solution by acidification with glacial acetic acid. However, if the amino group was present, addition of sodium acetate or ammonium chloride proved adequate to cause separation of the dyestuff. Digestion of the resultant suspension on a steam-bath for one hour usually caused the solid to coagulate and to filter more readily. Glacial acetic acid and ethyl acetate were used with some success as solvents for purification of the azo compounds; however, because of the large solubility of the dyes in pyridine, the best medium for crystallization was pyridine–water.

The structure of each of the azo derivatives is predicated upon the usual tendency exhibited in coupling by the respective phenol, amine or aminosulfonic acid. Physical and analytical data for these azo derivatives are listed in Table II.

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(18) Chilling the solution caused formation of a flocculent precipitate, but addition of the nitrite solution produced separation of a much greater amount of flocculent material.

[CONTRIBUTION FROM THE DEPARTMENT OF MICROBIOLOGICAL CHEMISTRY, SHARP AND DOHME DIVISION, MERCK & CO., INC.]

Biotin *l*-Sulfoxide. I. The Occurrence of a Previously Unrecognized Form of Biotin in Certain Fermentation Sources

BY LEMUEL D. WRIGHT AND EMLEN L. CRESSON

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Microbiological evidence is presented for the existence of a previously unrecognized form of biotin in certain mold filtrates where growth had taken place in the presence of added pimelic acid. The growth-promoting material has been tentatively named AN factor because it was first encountered in culture filtrates of *Aspergillus niger*. AN factor has a characteristic spectrum of microbiological activity. It may be distinguished from biotin and desthiobiotin and less readily from biocytin by paper chromatography. AN factor is more labile than biotin to both acid and alkali. It migrates on paper electrophoresis as a monocarboxylic acid. The factor may be reduced with zinc and hydrochloric acid or aluminum and sodium hydroxide to biotin and with Raney nickel to desthiobiotin. It combines with avidin.

The addition of pimelic acid or higher homologs to an *Aspergillus niger* fermentation was found by Eakin and Eakin to be associated with an increase in the biosynthesis of "biotin" as determined microbiologically with *Saccharomyces cerevisiae*.[1] Concurrently the nutritive requirement of *Corynebacterium diphtheriae* for pimelic acid[2] was found by du Vigneaud, *et al.*,[3] to be satisfied equally well with much smaller amounts of biotin indicating that pimelic acid probably is a precursor of biotin for this organism. Subsequent microbiological studies of Tatum[4] have furnished evidence that a factor formed from pimelic acid by certain molds is des-

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