Summary

Diene hydrocarbons of the formulas $CH_2{=}CR{-}CH{=}CH_2$ and/or $CH_2{=}C{=}CHCH_2R$ are obtained by the action on chloro-4-butadiene-1,2 of RMgX where R is methyl, *n*-butyl, *n*-heptyl, phenyl and benzyl. Some polymers and derivatives are described.

WILMINGTON, DELAWARE RECEIVED DECEMBER 20, 1932
PUBLISHED JULY 6, 1933

[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY OF THE UNIVERSITY OF CHICAGO]

Chemical Studies of the Mechanism of the Narcosis Induced by Hypnotics. I. The Synthesis of Colored Derivatives of Phenobarbital[1]

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In an effort to throw light upon the fundamental mechanism of the narcosis induced by hypnotics, an extensive program of research[2] has been undertaken at the instigation and under the direction of two of the authors. The end in view for the work discussed in the present paper has been the modification of the molecular structure of the synthetic hypnotic phenylethylbarbituric acid, or phenobarbital, $(C_6H_5)(C_2H_5)\underline{CCONHCONHC}{=}O$, in such a way as to give it color. It is hoped that some dye derivatives of phenobarbital may be found in this way which retain in their molecules the pronounced hypnotic effect of phenobarbital. It has been reasoned that, should such products be obtained, their color may render them useful for a study of the action of hypnotics in the cells of the brain and nerves, where the chief action of such sedatives occurs. The problem is a complex one: to be of any use for the purpose named the dye derivatives of phenobarbital must be, first of all, hypnotics; further, they must possess the property of staining nerve tissues selectively, a behavior by no means universal among dyes.

It is obvious that the pursuance of our program to its logical conclusion will necessitate the coöperation of chemists with pharmacological and histological experts. The present paper discusses the first investigations undertaken, which have been largely chemical. The dyes so far prepared by us have been studied pharmacologically by Dr. A. L. Tatum, Professor of Pharmacology at the University of Wisconsin.

Probably the first use of dye derivatives of drugs as an aid to the study of the mechanism of drug action was made by Ehrlich and Einhorn,[3] who

(1) The work here described forms part of the dissertation of John H. Shroyer, presented in partial fulfilment of requirements for the doctorate degree at the University of Chicago.

(2) The Julius Stieglitz Research Fund for Chemistry Applied to Medicine, made available by the Chemical Foundation, is supporting the main part of this work.

(3) Ehrlich and Einhorn, *Ber.*, **27**, 1872 (1894).

converted cocaine into a number of azo dyes. Only one of these was found to retain the anesthetic effect of cocaine. More recently Fulton[4] converted procaine, a synthetic substitute for cocaine, into a series of azo derivatives and found a very few of these to retain the local anesthetic action of the parent substance. Fulton then made an interesting histological study of the action of these dyes in the tissues, and arrived at some interesting conclusions. The work of Ehrlich, Einhorn and Fulton is of special value for its suggestiveness, and it seems likely that the basic principle of such study is a sound one, capable of successful application with many drugs other than local anesthetics.

Four dye derivatives of phenobarbital have so far been synthesized by the coupling of diazotized *m*-aminophenobarbital in turn with β-naphthol, resorcinol, salicyclic acid and tyrosine, namely, 5-β-naphthol-*m*-azophenyl-5-ethylbarbituric acid, $(HOC_{10}H_6N{=}NC_6H_4)(C_2H_5)\underline{CCONHCONHCO}$, 5-resorcinol-*m*-azophenyl-5-ethylbarbituric acid, $((HO)_2C_6H_3N{=}NC_6H_4)(C_2H_5)\underline{CCONHCONHCO}$, 5-salicylo-*m*-azophenyl-5-ethylbarbituric acid, $((HO)(COOH)C_6H_3)(C_2H_5)\underline{CCONHCONHCO}$, and 5-tyrosine-*m*-azophenyl-5-ethylbarbituric acid, $((HO)(CH_2CHNH_2COOH)C_6H_3N{=}NC_6H_4)(C_2H_5)\underline{CCONHCONHCO}$.

It will be noted that all of these compounds are acid, rather than basic, dyes. This was planned deliberately with two facts in mind. (1) All of the hypnotic cyclic ureides are weak acids and it was considered important to duplicate this behavior in dye-ureides. (2) The efficient hypnotics are fat soluble, this property being a requisite for solubility in the nerve tissues, which contain large amounts of fat-like substances. According to Michaelis,[5] fat-soluble dyes must have in their molecules an hydroxyl group attached to the ring in ortho position to the azo group.

Reports from Professor Tatum concerning the physiological effects of the four dyes prepared by us indicate that the modifications of the phenobarbital molecule in such a way as to give it color as so far accomplished have canceled the hypnotic effect of the drug. One of the authors is now working on a new series of compounds, giving special attention to the maintenance of the hypnotic effect in the dye derivatives. For example, diazotized aminophenobarbital is to be coupled with aminophenobarbital. The product will be an azo dye with two hypnotic groups. Should it prove to have the desired hypnotic effect, but lack the power to penetrate the fatty constituents of the nerve tissues, this lack might be supplied, in accordance with the Michaelis theory, by the coupling of the diazotized aminophenobarbital with hydroxyphenobarbital to form an acid azo dye. The work will be continued along these and related lines.

(4) Fulton, *Am. J. Physiol.*, **57**, 153 (1921).
(5) Michaelis, *Arch. path. Anat. (Virchow's)*, **164**, 263 (1901).

Experimental

(1) **5-*m*-Nitrophenyl-5-ethylbarbituric Acid.**—Phenobarbital was nitrated first by Ranwez[6] and later by Adams.[7] These workers offer no direct proof of the position of the nitro group on the benzene ring. Adams assigns it to the para position in the product which he isolated, apparently because he found that the nitration of methyl ethyl phenylethylmalonate, $(C_6H_5)(C_2H_5)C(COOCH_3)(COOC_2H_5)$, under conditions similar to those used by him for the nitration of phenobarbital produces methyl ethyl *p*-nitrophenylethylmalonate. The present authors obtained, upon nitration of phenobarbital under the conditions specified by Adams, a 55% yield of 5-*m*-nitrophenyl-5-ethylbarbituric acid of m. p. 279–280° (corr.).[8]

(2) **5-*m*-Aminophenyl-5-ethylbarbituric Acid.**—This base was prepared by Adams' method[7] of catalytic reduction of the nitro compound. A 90% yield of amine melting at 207.5–208.5° (corr.) was obtained by us.

(3) **5-β-Naphthol-*m*-azophenyl-5-ethylbarbituric Acid.**—To prepare this dye, the amine of phenobarbital (1.2 g.) was diazotized by being first suspended in 50 cc. of water containing 5 cc. of concd. hydrochloric acid and then treated slowly at 0° with sodium nitrite (0.4 g.) in 15 cc. of water. The solution of the diazotized amine obtained in this manner was treated with urea to destroy the excess of nitrous acid and then poured into a cold solution of 95% alcohol (100 cc.) containing 0.72 g. of β-naphthol. Aqueous potassium hydroxide (20%) was added to the reaction mixture until it was just acid to litmus. When this solution was stirred for two hours at a temperature below 5° an orange-red precipitate formed. The reaction mixture, brought to room temperature, was then poured into 150 cc. of water containing 5 cc. of concd. hydrochloric acid and the stirring was continued for ten minutes. The precipitated dye was brought upon a filter, washed with water and dried. It was purified (1) by solution in concentrated potassium hydroxide and reprecipitation with acid, and (2) by solution in pyridine and reprecipitation by the addition of 3–5 volumes of methyl alcohol. A yield of 95% of the theoretical was obtained. The orange crystalline compound decomposes at approximately 302° (corr.).

Anal. Calcd. for $C_{22}H_{18}O_4N_4$: C, 65.67; H, 4.50; N, 13.93. Found: C, 65.56, 65.49; H, 4.51, 4.52; N, 13.85, 13.95.

(4) **5-Resorcinol-*m*-azophenyl-5-ethylbarbituric Acid.**—*m*-Aminophenobarbital (2.5 g.) was diazotized as previously described. The acid solution of the diazotized base was then added slowly to 150 cc. of a well-cooled solution of 95% alcohol containing 1.1 g. of resorcinol and 8.5 g. of potassium hydroxide, and a further amount of alkali was added to make the reaction mixture just acid to litmus. The mixture was kept below 5°, stirred for six hours, and was then treated with 2 cc. of concentrated hydrochloric acid and 250 cc. of water and allowed to stand overnight. Orange crystals formed which contained alcohol of crystallization. These were collected and purified by being dissolved in alkali and reprecipitated by acid. The dye was further purified by solution in boiling acetic acid. Addition of water to this solution to turbidity, and slow cooling of the solution, led to the deposition of red needles. These were collected, washed with water, dried and analyzed. The yield was 3 g. or 83%. The dye decomposes at about 248–250° (corr.).

Anal. Calcd. for $C_{18}H_{16}O_5N_4$: C, 58.70; H, 4.35; N, 15.22. Found: C, 58.30, 58.36; H, 4.49, 4.48; N, 15.00, 15.07.

(5) **5-Salicylo-*m*-azophenyl-5-ethylbarbituric Acid.**—The diazotization of *m*-amino-

(6) Ranwez, *J. pharm. Belg.*, **6**, 410, 501 (1924).

(7) Adams and Bousquet, THIS JOURNAL, **52**, 224 (1930).

(8) The proof of the identity of the product obtained by us in the nitration of phenobarbital was brought by M. M. Rising and Alan Pierce and will be stated in detail in an early issue of THIS JOURNAL.

phenobarbital (1.2 g.) was carried out in the manner described and the solution of the diazotized amine was added to a well-cooled solution of salicylic acid (0.7 g.) dissolved in 100 cc. of a 6% sodium carbonate solution. The reaction mixture was stirred for two hours at a temperature below 5°, and then was allowed to stand overnight at room temperature. The addition of a small excess of concd. hydrochloric acid caused the precipitation of a cinnamon colored dye. This was collected on a filter and purified by solution in 6% sodium carbonate solution and reprecipitation with acid. The weight of pure dye obtained was 1.5 g., a 79% yield. The compound decomposes at approximately 250–251° (corr.).

Anal. Calcd. for $C_{18}H_{16}O_6N_4$: C, 57.58; H, 4.04; N, 14.14. Found: C, 57.57, 57.63; H, 4.24, 4.21; N, 13.97, 13.98.

(6) **5-Tyrosine-*m*-azophenyl-5-ethylbarbituric Acid.**—Aminophenobarbital (2.5 g.) was diazotized as previously described. The solution of the diazotized base was added to 100 cc. of a cold solution of 9% potassium hydroxide in which was dissolved 1.81 g. of tyrosine. The reaction mixture was kept faintly alkaline to litmus and was stirred for five hours at a temperature below 5°. The mixture was then made just alkaline to litmus and allowed to stand overnight. Precipitation of the dye occurred; the substance was brought upon a filter, washed and dried. Purification of the compound was accomplished by its solution in hot 95% ethyl alcohol from which it was precipitated when the alcohol solution was poured into four volumes of water. The yield of pure dye was 1 g., a 23% yield. The compound is light brown, undergoes a color change at 200° (corr.) and decomposes at 216–222° (corr.).

Anal. Calcd. for $C_{21}H_{21}O_6N_5$: C, 57.40; H, 4.78; N, 15.95. Found: C, 56.70, 56.73; H, 4.49, 4.47; N, 15.66, 15.84.

Summary

1. A plan for the study of the mechanism of the narcosis induced by hypnotics is outlined. This plan involves the application of chemical, pharmacological and histological methods.

2. The synthesis of colored hypnotics has been attempted.

3. Four dye derivatives of phenobarbital have been prepared.

CHICAGO, ILLINOIS RECEIVED JANUARY 16, 1933
PUBLISHED JULY 6, 1933