[CONTRIBUTION FROM THE LABORATORIES OF THE MT. SINAI HOSPITAL, NEW YORK]

OPTICALLY ACTIVE 5,5′-DISUBSTITUTED HYDANTOINS[1]

BY HARRY SOBOTKA, MARJORIE F. HOLZMAN AND JOS KAHN[2]

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In 1916, 5,5′-phenylethylhydantoin was introduced under the trade name "nirvanol" as a hypnotic and sedative. This drug shows certain advantages in comparison with the related disubstituted barbituric acids, but its wider application has been curtailed by its toxic properties manifested by what is commonly referred to as "nirvanol disease." Several attempts have been made to modify and improve phenylethylhydantoin by replacement of the substituting groups. Thompson, Bedell and Buffett[3] prepared methyl-, propyl-, isopropyl-, butyl-, and isobutyl-phenylhydantoin. Hill[4] prepared various furoyl-alkylhydantoins. Herbst and Johnson[5] quite recently published data on benzyl- and phenethyl-alkylhydantoins. Shonle[6] includes some of Herbst and Johnson's hydantoins in a general discussion of hypnotics.

It seems to have escaped the attention of those working with hypnotics of the hydantoin group, that 5,5′-disubstituted hydantoins contain an asymmetric carbon atom and that these synthetic products are racemic mixtures.

Optically active 5-monosubstituted hydantoins have been prepared by Lippich[7] from amino acids plus urea or cyanate. Dakin[8] studied the spontaneous racemization of such hydantoins during synthesis and found that no racemization took place in the case of levo-isovaline. This amino acid is the only naturally occurring one which gives rise to a disubstituted hydantoin. The dextrorotatory ethylmethylhydantoin of Dakin is the first asymmetric disubstituted hydantoin to be recorded.[9]

We succeeded in separating racemic phenylethylhydantoin into its pure optically active components by means of brucine, which forms a less soluble salt with the dextro hydantoin than with the levo isomer. The optical rotation of the enantiomers is $\pm 123°$, their melting point 237°. Dextro- and levo-phenylethylhydantoin can be hydrolyzed to dextro- and levo-

[1] The expenses of this investigation were defrayed by a grant obtained through the kindness of Dr. B. Schick.

[2] Hernsheim Research Fellow, 1931.

[3] Thompson, Bedell and Buffett, THIS JOURNAL, **47**, 874 (1925).

[4] Hill, Abstract, Meeting of American Chemical Society, Buffalo, September, 1931, Biochemical Section.

[5] Herbst and Johnson, THIS JOURNAL, **54**, 2463 (1932).

[6] Shonle, *Ind. Eng. Chem.*, **23**, 1104 (1931).

[7] Lippich, *Ber.*, **41**, 2974 (1908).

[8] Dakin, *Am. Chem. J.*, **44**, 59 (1911).

[9] A substance which may be classified as an optically active disubstituted hydantoin is the spiro-hydantoin of Pope and Whitworth, *Chem. & Ind.*, **49**, 748 (1930).

phenylethylglycine. This pair of amino acids may also be obtained from racemic phenylethylglycine[10] through optical resolution of its formyl compound with quinine. The optically active amino acids in turn may be converted into the corresponding hydantoic acids and hydantoins. Since all these reactions are accomplished without any intercurrent racemization, the optical forms of phenylethylhydantoin are individually accessible from racemic phenylethylglycine as starting point.

The derivatives of phenylethylglycine display a noteworthy resistance against racemization. Dakin explained this stability in the ethylmethyl series by the absence of a hydrogen atom on the asymmetric carbon atom, while derivatives of monosubstituted aminoacetic acids may lose their optical activity through enolization. We believe that Dakin's explanation also holds in the present instance.

The sense of rotation remained unchanged throughout the reactions in the phenylethyl series. This is in contrast to the transition of levo-isovaline into dextrorotatory ethylmethylhydantoin. We assume, therefore, that the configuration on the asymmetric carbon atom in levo-ethylmethylglycine (isovaline) is analogous to that in dextro-phenylethylglycine, as both become more dextrorotatory by closure of the hydantoin ring. Following Levene's conceptions and experiences in homologous series, one may frequently draw conclusions as to the relative order of the substituents around the central carbon atom; but too little is known regarding the influence of the phenyl group to refer definitely the constellation of a phenylethyl and a methylethyl derivative to each other.

Other new hydantoins were prepared containing as substituents allyl-isopropyl (as in "allonal") and bromoallyl-isopropyl (as "noctal"). Sufficient quantities of allyl-isopropylhydantoin were available to attempt its optical resolution. The dextrorotatory product isolated had a specific rotation of $+7.5°$. Owing to the poor yield, the question could not be settled whether or not this product represents the pure dextro form; however, on theoretical grounds, no high rotation would be expected in this compound.

Experimental

Resolution of Racemic Phenylethylhydantoin into its Optically Active Components.—Equivalent amounts of 5,5'-phenylethylhydantoin (1 part) m. p. 198°, and of brucine (2.3 parts) are dissolved in absolute alcohol (10–15 parts). On standing, rosets of crystals separate (about 1.6 parts), consisting of the brucine salt of dextro-phenylethylhydantoin. An excess of dilute sulfuric acid is added to a solution of these crystals in absolute alcohol and sufficient water is added to reduce the alcohol content to 10%. The free dextro-phenylethylhydantoin crystallizes in white flaky platelets. They are recrystallized several times from dilute alcohol until free from brucine. The pure substance melts sharply at 237°, and the purest specimens showed a rotation $[\alpha]_D$ $+123°$ in alcoholic solution (0.5514 g. in 25 cc., 2-dm. tube, α_D $+5.36°$). The specific

[10] Jawelow, *Ber.*, **39**, 1195 (1906).

rotation in alkaline aqueous solution was +169° (0.2521 g. in 25 cc., 2-dm. tube, α_D +3.40°).

Anal. Calcd. for $C_{11}H_{12}O_2N_2$ (204.10): C, 64.67; H, 5.93; N, 13.72. Found: C, 64.09; H, 6.18; N, 13.96.

The mother liquor from the brucine-dextro-hydantoin is carefully acidified until it becomes cloudy. On standing, the levo-phenylethylhydantoin separates in solid crystals. It is purified in the same manner as its enantiomer until its melting point reaches 235–237°; $[\alpha]_D$ −121° (0.2562 g. in 25 cc. alcohol, 2-dm., α_D −2.49°); −167° in aqueous alkali (0.2981 g. in 25 cc., 2-dm., α_D −4.00°).

Anal. Calcd. for $C_{11}H_{12}O_2N_2$ (204.10): C, 64.67; H, 5.93; N, 13.72. Found: C, 64.04; H, 6.11; N, 13.74.

Racemic phenylethylglycine (*dl*-α-amino-α-phenylbutyric acid)[5,10] was prepared by hydrolysis of racemic phenylethylhydantoin with 75% sulfuric acid for forty-eight hours. The sulfate of the amino acid is obtained in crystalline form and is purified by reprecipitation from alcoholic solution with ether. It may be used directly for formylation (see below). The free amino acid is characterized by its beautiful copper salt, which crystallizes in dark blue polygonal platelets with 2 moles of crystal water per atom of copper. On standing over phosphorus pentoxide, it is converted into the lavender colored anhydrous salt.

Anal. Calcd. for $(C_{10}H_{12}O_2N)_2Cu\cdot 2H_2O$ (455.81): N, 6.16; Cu, 15.14. Found: N, 5.95; Cu, 15.30.

Racemic Formylphenylethylglycine.—Five grams of amino acid sulfate is refluxed for three hours on the water-bath with 2 g. of sodium formate and 10 g. of 95% formic acid. The formic acid is removed at reduced pressure, the temperature being kept below 55°. Fresh formic acid is added two or three times and the reaction product recrystallized from water; m. p. 193°.

Anal. Calcd. for $C_{11}H_{14}O_4N$ (224.13): N, 6.28. Found: N, 5.64. No amino nitrogen.

After many trials it was found that the resolution of this racemate can best be accomplished with quinine in 20% alcohol. The salt of the dextro-formyl compound is the less soluble. The alkaloid is removed in aqueous solution by alkali, and precipitation of the supernatant with acid yields the free **dextro-formylphenylethylglycine,** m. p. 212°, $[\alpha]_D$ +126° (0.2510 g. in 25 cc. aqueous alkali, α_D in 2-dm. tube +2.53°). An identical product with $[\alpha]_D$ +126° (0.2524 g., 25 cc., 2-dm. tube, α_D +2.55°) is obtained by formylation of dextro-phenylethylglycine. This confirms the optical purity of the compound.

On the other hand, deformylation by means of dilute sulfuric acid results in the formation of **dextro-phenylethylglycine,** which is found identical with the dextrorotatory amino acid as obtained by hydrolysis of the dextro-hydantoin; $[\alpha]_D$ +41° (0.1006 g. in 10 cc. aqueous alkali, 2-dm. tube, α_D +0.82°).

Anal. Calcd. for $C_{10}H_{13}O_2N$ (179.12): N, 7.82. Found: N, 8.25. Calcd. for anhydrous copper salt, $(C_{10}H_{12}O_2N)_2Cu$ (419.78): N, 6.69. Found: N, 6.66.

Levo-phenylethylglycine is likewise obtained from the respective hydantoin; $[\alpha]_D$ −41° (0.1014 g. in 10 cc. aqueous alkali, 2-dm. tube, α_D −0.83°).

Anal. Calcd.: N, 7.82. Found: N, 7.90.

Dextro-phenylethylglycine ethyl ester is prepared from the dextro amino acid by treatment with dry hydrogen chloride in ethyl alcohol for three hours. After removal of the alcohol *in vacuo,* the crystalline residue was washed with ether. The yield of the amino acid ester hydrochloride is increased by an additional precipitate from the ether

washings with petrolic ether. The free ester decomposed around 270°; $[\alpha]_D$ +36° (0.076 g. in 10 cc. water, 2-dm. tube, α_D +0.55°).

Anal. Calcd. for $C_{12}H_{17}O_2N$ (207.15): N, 6.76. Found: N, 6.89.

The syntheses of racemic, dextro- and levo-phenylethylhydantoin from the corresponding amino acids (or their ethyl esters) were usually carried out by means of urea. The rate of formation and of disappearance of the intermediary phenylethylhydantoic acid $(C_6H_5)(C_2H_5)C(COOH)NHCONH_2$ was studied in one case for the racemic series. Five grams of amino acid plus 17.5 g. of urea were dissolved in 25 cc. of water and heated on the water-bath. Five-cc. samples were withdrawn at intervals and cooled. In the first samples, no hydantoin had yet formed and the hydantoic acid was isolated from the clear solution by precipitation with sulfuric acid. The hydantoin started to crystallize in the fifth hour and the synthesis was complete in forty-eight hours.

Hours	1	2	3	5	16	48
Hydantoic acid in 5 cc. of supernatant, mg	211	283	277	256	145	none

The phenylethylhydantoic acids have no definite melting points but liquefy with loss of water between 170 and 180°. They solidify again at higher temperature and then approach the melting point of the hydantoin; $[\alpha]_D$ +60° (and −62°) (0.2411 g. and 0.2532 g. in 25 cc. aqueous alkali, 2-dm. tube, α_D +1.15° and −1.25°, resp.).

5,5'-Phenylethyl-3-phenyl-2-thiohydantoin was synthesized from phenylethylglycine and phenylthiourea; m. p. 143°.

Anal. Calcd. for $C_{17}H_{16}ON_2S$ (296.18): N, 9.45. Found: N, 9.12.

Amide of Isopropylcyanoacetic Acid (Isopropylmalonic Acid Amide Nitrile).—Equivalent amounts of the sodium cyanoacetic ethyl ester and 2-iodopropane are refluxed in alcohol for several hours. After dilution with water, the isopropyl cyanoacetic ester is separated and distilled *in vacuo* at 25°.[11] The colorless liquid is shaken with concentrated aqueous ammonia for several days. Isopropylcyanoacetamide is obtained and recrystallized from benzene; m. p. 124°.[12]

Anal. Calcd. for $C_6H_{10}ON_2$ (126.10): N, 21.21. Found: N, 21.65.

Its solution is treated with allyl iodide, $CH_2{=}CHCH_2I$ in the presence of sodium ethylate for several hours. The reaction mixture is taken up in ether and the washed and dried ethereal solution evaporated to dryness.

The **isopropylallylcyanoacetamide** is recrystallized from small amounts of amyl alcohol; m. p. 87°.

Anal. Calcd. for $C_9H_{14}ON_2$ (166.13): N, 16.86. Found: N, 17.20.

This amide is added to a solution of potassium hypobromite in excess alkali and after two hours of standing it is heated on the water-bath for three hours to finish the reaction. Upon acidification, **isopropylallylhydantoin** of m. p. 187° crystallizes.

Anal. Calcd. for $C_9H_{14}O_2N_2$ (182.13): C, 59.28; H, 7.69; N, 15.38. Found: C, 58.90; H, 7.63; N, 15.04.

This product was also synthesized, by reversing the order of introduction of the alkyl groups, via the ester and the amide[13] of allylcyanoacetic acid. This preparation showed identical properties.

While brucine, cinchonine or Betti's base proved ineffective[14] in the resolution of

[11] Hessler, THIS JOURNAL, **35**, 1992 (1913).

[12] Henry, see Beilstein, fourth ed., Vol. II, p. 669.

[13] Henry, see Beilstein, fourth ed., Vol. II, p. 776.

[14] "Organic Syntheses," John Wiley and Sons, Inc., New York, 1931, Vol. XI, p. 60.

this racemic hydantoin, **dextrorotatory allylisopropylhydantoin** could be obtained through the quinine salt; $[\alpha]_D$ +7.0° (0.1870 g. in 10 cc. alcohol, 2-dm. tube, α_D +0.26°). Owing to the poor yield, the question could not be settled whether this product represented the pure dextro form.

β-Bromoallylisopropylhydantoin, m. p. 214–215°, was obtained in unsatisfactory yield from isopropylcyanoacetamide plus propylene dibromide, $CH_2{=}CBrCH_2Br$, and oxidative rearrangement with potassium hypobromite.

Pharmacological and clinical experiments with these substances will be reported in detail elsewhere.[15] It was found in various animal species and by various methods of administration, that the Min. Eff. Dose and the Min. Lethal Dose of the dextro substance are 25 to 50% higher than for the levo substance, leaving the racemate in the middle (see table below).

While this difference is relatively insignificant in view of the therapeutic application, a striking difference was discovered between the two isomers as concerns the causation of the "nirvanol disease" upon oral administration. The incidence of "nirvanol disease" in 25 cases treated with an average aggregate dose of 4.2 g. of the racemate was 21 cases or 84%; in 14 cases, treated with an average amount of 2.1 g. only of the levorotatory drug, 9 cases or 64.5%. In a series of 18 cases which received an average of 2.8 g. each of dextro-phenylethylhydantoin, 5 cases (28%) developed the disease, while in another series of 15 adults who received much higher amounts of the dextro form, only 2 (13.5%) were affected.

These observations shed new light on the mechanism, allergic or otherwise, by which drugs and synthetic chemicals in general produce reactions in the human body.

Animal	Dose	Route of administration	Dextro	Racemic	Levo
			milligrams		
Rabbit	M. L. D.	Subcut.	350	250	250
Guinea pig	M. L. D.	Subcut.	250	200	200
Rat	M. E. D.	Subcut.	100	80[a]	80
Dog	M. E. D.	Perorally	100	<100	70
Pigeon	M. E. D.	Intraven.	75	[b]	50

[a] Allylisopropylhydantoin, 200 mg. [b] β-Bromoallylisopropylhydantoin, 40 mg.

We wish to thank Priv.-Doz. Dr. E. Rothlin, Basel, for giving us the results of his animal experiments with these substances.

Summary

5,5′-Phenylethylhydantoin was separated into its pure optically active forms. These optical antipodes were also independently synthesized from the optically active phenylethylglycine obtained by optical resolution of its racemate. The stability against racemization and the sense of rotation of these derivatives are discussed.

A brief report on the pharmacological and clinical value of dextro- and levo-phenylethylhydantoin shows that the substances differ slightly in

[15] Sobotka, Peck and Kahn, *J. Pharm. Exp. Ther.*, in press; Schick, Sobotka and Peck, *Am. J. Dis. Child.*, in press.

hypnotic effect. The dextro form, however, is three times less toxic as judged by the greatly reduced incidence of "nirvanol disease."

Allylisopropylhydantoin and β-bromoisopropylhydantoin were prepared and tested.

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OCCURRENCE OF DECOMPOSITION PRODUCTS OF CHLOROPHYLL. I. DECOMPOSITION OF CHLOROPHYLL IN THE DIGESTIVE SYSTEM OF THE COW

BY PAUL ROTHEMUND AND O. L. INMAN

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The study of natural porphyrins, especially copro-, uro- and ooporphyrin, was of greatest importance in ascertaining the constitution of hemin. Phylloerythrin, a biological decomposition product of chlorophyll, is for similar reasons of particular interest for studies in the chlorophyll series. L. Marchlewski[1] discovered it in the fresh feces of cows fed on green food. In the same year Loebisch and Fischler[2] isolated it from ox bile under the name bilipurpurin. Marchlewski[3] proved the identity of these two substances and their identity with MacMunn's cholehematin. Marchlewski also showed that phylloerythrin is derived from chlorophyll and not from hemoglobin. The porphyrin nature of phylloerythrin was demonstrated by H. Fischer and H. Hilmer,[4] who also proved D. Kémeri's porphyrin from human feces to be identical with phylloerythrin. By chemical methods H. Fischer and co-workers succeeded in preparing the substance from chlorophyll derivatives such as chlorophyllide $a + b$, pheophytin $a + b$, pheophorbide a, methylpheophorbide a, pheoporphyrin a_5.[5]

In a preliminary note[6] we announced that phylloerythrin occurs in the stomachs of herbivorous animals (cow and sheep). It has the formula I according to H. Fischer and he considers this structure of fundamental importance for the understanding of the nature of chlorophyll a. The study of decomposition products of chlorophyll in the animal body presents many possibilities. (1) Waste products can be examined. This method led to the discovery of phylloerythrin.[1] (2) Examination of the contents

[1] Marchlewski, *Bull. intern., acad. polonaise des sciences et des lettres*, **A**, 638–642 (1903).

[2] Loebisch and Fischler, *Monatsh.*, 335–350 (1903).

[3] Marchlewski, *Bull. soc. chim. biol.*, **6**, 464–472 (1924).

[4] Fischer and Hilmer, *Z. physiol. Chem.*, **143**, 1–8 (1925).

[5] H. Fischer and O. Süs, *Ann.*, **482**, 225–232 (1930); H. Fischer, O. Moldenhauer and O. Süs, *ibid.*, **486**, 107–177 (1931).

[6] Inman and Rothemund, *Science*, 221 (1931).