604. *Aryl-2-halogenoalkylamines. Part XIX.* Some* NN-*Di*-2-*chloroethylamino-phenyl- and -phenylalkyl-hydantoins and Related Amino-acids.*

By T. A. Connors, W. C. J. Ross, and J. G. Wilson.

5-(*m*-Di-2-chloroethylaminophenyl)-, 5-(*p*-di-2-chloroethylaminophenyl)-, and 5-(4-di-2′-chloroethylaminobenzyl)hydantoin, their 5-methyl derivatives, and also 5-(*p*-di-2-chloroethylaminophenyl-ethyl- and -propyl)hydantoin have been prepared. Several of the hydantoins have been converted into the corresponding amino-acids. A new route to the tumour-growth inhibitor " merphalan " (" sarcolysin ") is described and the preparation of its ortho-isomer is reported.

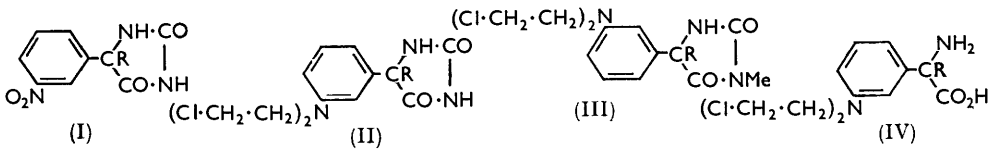
In continuation of our studies on cytotoxic compounds with latent activity, that is, compounds whose action is promoted by a process known to occur *in vivo*, we have examined some *NN*-di-2-chloroethylamino-derivatives of aryl- and arylalkyl-hydantoins. It has been established that hydantoins can be converted into amino-acids in the organism [1]

* Part XVIII, *J.*, 1956, 1724.

[1] Kozelka and Hine, *J. Pharm. Exp. Therap.*, 1943, **77**, 175.

and hence the above hydantoins would be precursors of the series of active chloroethylaminoarylamino-acids.[2,3] In a number of cases it has been possible to obtain the amino-acids from the hydantoins by hydrolysis with concentrated acid.

m-Nitrophenylglycine readily yielded the corresponding hydantoin (I; R = H) by way of the hydantoic acid, and catalytic reduction then gave the amine which was converted into 5-(*m*-di-2-chloroethylaminophenyl)hydantoin (II; R = H) by methods already described in this series. This hydantoin afforded the glycine derivative (IV; R = H) when heated at 170° with concentrated hydrochloric acid.



N-Methylation often reduces the stability of heterocyclic structures and this should lead to the more ready release of an amino-acid. Accordingly the hydantoin (II; R = H) was converted into the 3-methyl derivative (III; R = H) by treatment with diazomethane, the position of the methyl group being confirmed by the production of the amino-acid (IV; R = H) on hydrolysis. 5-*p*-Di-2-chloroethylaminophenylhydantoin was similarly prepared from the hydantoin obtained by the Bucherer–Bergs method from *p*-aminobenzaldehyde. The corresponding amino-acid has already been described.[3]

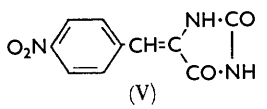
The 5-methylhydantoin derivatives (II and III; R = Me) were of interest for two reasons. 5,5-Disubstituted hydantoins have anticonvulsant activity and the incorporation of such a structure into an " aromatic nitrogen mustard " might reduce the toxicity which involves, *inter alia*, induction of convulsions. Secondly, α-methylated amino-acids are reported to be concentrated within cancer cells,[4] and the hydantoins (II and III; R = Me) might therefore be precursors of amino-acids with useful properties. *m*-Nitroacetophenone afforded the hydantoin (I; R = Me) which was converted into the di-2-chloroethylamino-compound (II; R = Me), its *N*-methyl derivative (III; R = Me), and the amino-acid (IV; R = Me) in the usual manner. *p*-Nitroacetophenone was converted into 5-*p*-di-2-chloroethylaminophenyl-5-methylhydantoin by a similar sequence of reactions.

p-Nitrobenzylidenehydantoin has been prepared by condensing *p*-nitrobenzaldehyde with hydantoin.[5,6] In this reaction it has been found to be important to avoid overheating, otherwise a high-melting polymer is formed. Hydrogenation of the hydantoin (V) in presence of palladium–charcoal in dimethylformamide, Raney nickel in alkaline ethanolic solution, or Adams platinum catalyst in acetic acid involved only the nitro-group, giving 5-4′-aminobenzylidenehydantoin (VI). Complete reduction to the benzyl derivative (VII) could only be achieved in low yield, by red phosphorus in concentrated hydrochloric acid. The required 5-4′-aminobenzylhydantoin was more conveniently prepared by catalytic reduction of the nitrobenzylhydantoin which was readily obtained from *p*-nitrophenylalanine.

5-(4-Di-2′-hydroxyethylaminobenzyl)hydantoin (VIII; R = H, X = OH) was the main product from the reaction of ethylene oxide with the amine (VII) but in one run a trihydroxyethyl derivative, probably (IX), was formed. 5-(4-Di-2′-chloroethylaminobenzyl)hydantoin (VIII; R = H; X = Cl) and its *N*-methyl derivative (VIII; R = Me, X = Cl) were readily prepared, and acid hydrolysis of these afforded a new route to the highly active tumour-growth inhibitor, *p*-di-2-chloroethylamino-DL-phenylalanine[2] (X)

[2] Bergel and Stock, *J.*, 1954, 2409.
[3] Davis, Roberts, and Ross, *J.*, 1955, 890.
[4] Christensen, A Symposium on Amino-acid Metabolism, McCollum-Pratt Institute, pp. 63—106, Baltimore, Maryland, 1954.
[5] Wheeler and Hofmann, *Amer. J. Chem.*, 1911, **45**, 568.
[6] Johnson and Brautlecht, *J. Biol. Chem.*, 1912, **12**, 175.

(" merphalan," " sarcolysin "). An alternative route to this compound, starting with condensation of *p*-di-2-chloroethylaminobenzaldehyde with hydantoin, could not be realised.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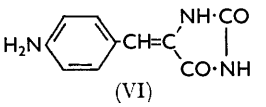
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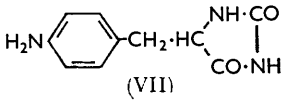
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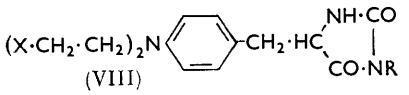
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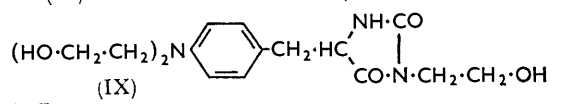
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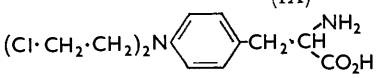
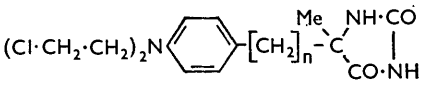

(X)

Three further 5-di-2-chloroethylaminoarylalkyl-5-methylhydantoins were prepared, starting from aminoarylalkyl methyl ketones, namely, the compounds (XI; $n = 1$—3).

The cyclic analogue (XII) was similarly prepared from 7-aminotetralone and hydrolysed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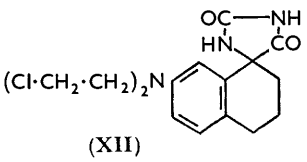
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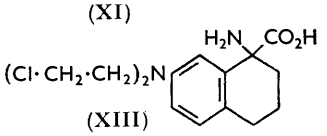
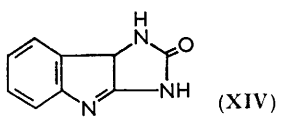

(XIII)


(XIV)

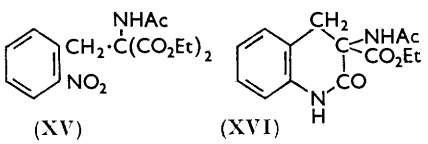
to the amino-acid (XIII). In this series of compounds intensive drying was often necessary in order to obtain an anhydrous specimen for analysis and occasionally water was retained even after such drying.

Few *ortho*-substituted di-2-chloroethylarylamines have been prepared so far because of the ready formation of unwanted cyclic intermediates (cf. ref. 7). Two attempts to make 5-(*o*-di-2-chloroethylaminophenyl)hydantoin were unsuccessful. In the first, *o*-aminobenzaldehyde was submitted to the Bucherer–Bergs process for making hydantoins and the product, which gave good analytical figures for the aminohydantoin, appeared to be devoid of amine reactions. By analogy with the formation of compound (XVIII) (see below) it was thought that the product might be the hydrate of the tricyclic compound (XIV): the tenacious retention of water in this series has already been mentioned. However, there were differences in the ultraviolet absorption spectra of the two compounds. When warmed with hydrochloric acid the compound was converted into a chlorine-free product, m. p. 240°, of lower nitrogen content but with similar light absorption; it is probably 5-*o*-hydroxyphenylhydantoin. An attempt to prepare 5-(*o*-di-2-hydroxyethylaminophenyl)hydantoin by the condensation of 5-*o*-chlorophenylhydantoin with diethanolamine was unsuccessful.

Cyclisation also precluded the use of two possible methods for the synthesis of *o*-di-2-chloroethylaminophenylalanine. Catalytic reduction of diethyl α-acetamido-2-nitrobenzylmalonate (XV) gave the lactam (XVI), and that of 5-2′-nitrobenzylhydantoin (XVII) gave the tricyclic compound (XVIII). 5-2′-Nitrobenzylhydantoin (XVII) was prepared by way of the hydantoic acid which in turn was obtained from the *o*-nitrophenylalanine formed by hydrolysis of the diester (XV). The product obtained by Bucherer and Lieb [8] by nitration of 5-benzylhydantoin must have been relatively impure. The

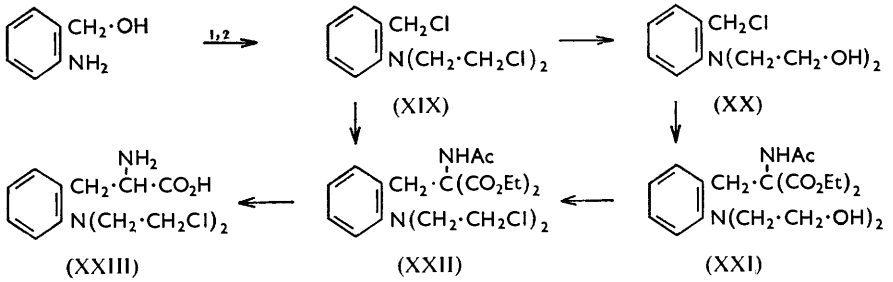
[7] Everett, Roberts, and Ross, *J.*, 1953, 2386.
[8] Bucherer and Lieb, *J. prakt. Chem.*, 1934, **141**, 5.

absorption spectrum of the reduction product from (XVII) (maxima at 280, 324, and 332 mμ) is consistent with its formulation as (XVIII) since it has a much higher extinction coefficient than would be expected for a benzylhydantoin. The formation of a dehydro-derivative of (XVIII) by reduction of 5-2′-nitrobenzylidenehydantoin with hydriodic acid and red phosphorus has been described by Kozak and Musial.[9]



(XV) (XVI) (XVII) (XVIII)

It was realised that the generation of an amino-group in a structure capable of cyclisation had to be avoided if the required *o*-compound was to be prepared. This could be achieved by the reaction sequence outlined below. It was originally intended to prepare the trichloro-compound (XIX) and to hydrolyse this in aqueous acetone to give the chloro-diol (XX), it being known [10] that *o*-substituted *NN*-di-2-chloroethylamines are



(XXIII) (XXII) (XXI)

1, Ethylene oxide; 2, phosphoryl chloride.

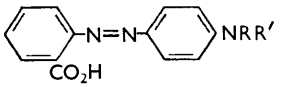
hydrolysed very readily under these conditions, whereas a benzyl chloride would not be reactive. Compound (XX) should yield the esters (XXI) and (XXII) in the usual manner. Since the chlorine atoms in the chloroethylamino-group in (XIX) would only be reactive under ionising conditions it was thought possible that, if one equivalent of the sodio-derivative was allowed to react with it in anhydrous ethanol, then the ester (XXII) might be formed directly. This reaction was successfully carried out. The evidence for exclusive reaction of the chloromethyl group is afforded by the behaviour of the derived amino-acid on paper chromatograms [the R_F values are similar to those of the *m*- and *p*-isomers of structure (XXIII)], and the fact that apart from unchanged material only one other product, namely (XXII), was detected in the reaction mixture. Acid-hydrolysis of the diester (XXII) yielded the required phenylalanine derivative (XXIII) in high yield.

Activity in the series of halogenoalkylamines was shown earlier [11] to depend on the presence of two alkylating groups in the molecule: all subsequent work with derivatives of aromatic amines has confirmed this. As a further test of this point we prepared mono-functional analogues of the two highly active " nitrogen mustards," γ-(*p*-*NN*-di-2-chloro-ethylaminophenyl)butyric acid (Chlorambucil) and (*NN*-di-2-chloroethylaminophenyl)-ethylamine. The *N*-2-chloroethyl-*N*-ethyl compounds were prepared from the corresponding amines, monoethylation being achieved by using Raney nickel and ethanol [12] and the chloroethyl group being subsequently introduced by established methods.

Biological Results.—Preliminary tests indicate that the majority of the 5-di-2′-chloro-ethylaminohydantoins and derived amino-acids now described are active inhibitors of the

[9] Kozak and Musial, *Bull. intern. Acad. polon.*, 1930A, 432.
[10] Ross, *J.*, 1949, 183.
[11] Haddow, Kon, and Ross, *Nature*, 1948, **162**, 824.
[12] Rice and Kohn, *J. Amer. Chem. Soc.*, 1955, **77**, 4052; Ainsworth, *ibid.*, 1956, **78**, 1635.

growth of the transplanted Walker rat carcinoma. Compounds (II and IV; R = H) are especially active; a single dose of 3 mg. per rat of either completely inhibits tumour-growth. 5- or 3-Methylation of the hydantoins greatly reduces activity and this is not due to any marked effect on the chemical reactivity of the chlorine atoms. The hydantoin (XII) and the corresponding amino-acid (XIII) are also highly active, the former having a pronounced effect on the growth of the more resistant Crocker sarcoma in mice.



(XXIV)

The special interest in di-2-chloroethylamino-derivatives of phenylalanine has been discussed in recent publications,[13] particularly with regard to the preparation of a compound in which the *para*-position is free for hydroxylation *in vivo*. Even in the *meta*-substituted derivative described in these publications hydroxylation in the *para*-position may be hindered by the presence of the bulky and flexible chloroethylamino-group. For this reason the less hindered *o*-di-2-chloroethylaminophenylalanine was prepared for test. The amino-acid (XXIII) caused complete inhibition of the growth of the Walker tumour at a dose of 0·1 mg. per rat, being more effective than either the *m*- or the *p*-isomer.

The monofunctional alkylating agent γ-(*p*-*N*-2-chloroethyl-*N*-ethylaminophenyl)-butyric acid, whilst at least as toxic as the difunctional analogue, Chlorambucil, had no effect on tumour growth. This supports the view that the action of carcinostatic agents is not merely one of general toxicity. An earlier example of this was afforded by compounds of structure (XXIV; R = Et, R′ = CH_2·CH_2Cl; and R = R′ = CH_2·CH_2Cl), where the monofunctional compound was at least five times as toxic as the difunctional derivative but only the latter inhibited tumour-growth. Another example, in the phenylalanine series, is given by Bergel and Stock.[14]

Experimental

M. p.s are corrected. Chromatograms were run on Whatman No. 1 paper in solvent *A*, butan-1-ol–ethanol–water–propionic acid (10 : 5 : 5 : 2), or *B*, water-saturated butanol. Absorption spectra were determined for ethanolic solutions.

5-m-*Nitrophenylhydantoin*.—*m*-Nitrophenylglycine [15] (4·05 g.) was dissolved in the minimum amount of boiling water and after the addition of potassium cyanate (2 g.) heated on a steam-bath for 30 min. Adding hydrochloric acid to the cooled solution precipitated the hydantoic acid, m. p. 186—188°. When this was heated at 100° in 20% aqueous hydrochloric acid for 30 min. the m-*nitrophenylhydantoin* (1·67 g.) was obtained. It formed yellow prisms, m. p. 220°, from water (Found: C, 48·8; H, 3·3; N, 18·8. $C_9H_7N_3O_4$ requires C, 48·9; H, 3·2; N, 19·0%).

5-m-*Aminophenylhydantoin*.—A solution of *m*-nitrophenylhydantoin (11·5 g.) in dimethylformamide (200 ml.) containing 5% palladium–charcoal (750 mg.) was shaken in hydrogen. When 3 mols. of hydrogen had been taken up the solution was filtered and evaporated under reduced pressure. After treatment with charcoal a hot aqueous solution of the residue deposited the *aminophenylhydantoin* as yellow prisms, m. p. 165—166° (Found: C, 55·4; H, 5·4; N, 21·3. Calc. for $C_9H_9N_3O_2$: C, 56·5; H, 4·8; N, 22·0. $C_9H_9N_3O_2,\frac{1}{4}H_2O$ requires C, 55·4; H, 4·9; N, 21·5%). It gave an *acetyl derivative*, prisms, m. p. 224—226° (from water, ethanol, or methanol) (Found: C, 56·7; H, 4·9; N, 18·1. $C_{11}H_{11}N_3O_4$ requires C, 56·7; H, 4·8; N, 18·0%).

5-(m-*Di*-2-*hydroxyethylaminophenyl*)*hydantoin*.—*m*-Aminophenylhydantoin (10·7 g.), ethylene oxide (28 ml.), acetic acid (3·4 ml.), and water (50 ml.) were stirred at room temperature for 18 hr. The resulting suspension was evaporated under reduced pressure and the residual solid recrystallised from water, giving a granular solid (9 g.), m. p. 90°, which rose to 129—130°

[13] Osdene, Ward, Chapman, and Rakoff, *J. Amer. Chem. Soc.*, 1959, **81**, 3100; Gram, Mosher, and Baker, *ibid.*, p. 3103.
[14] Bergel and Stock, *J.*, 1959, 90.
[15] Plöchl and Loë, *Ber.*, 1885, **18**, 1179.

after drying on a porous tile. Analysis indicated that the product was 5-(m-*di*-2-*hydroxyethylaminophenyl*)*hydantoin hemihydrate* (Found: C, 53·7; H, 6·1; N, 14·6. $C_{13}H_{17}N_3O_4,\frac{1}{2}H_2O$ requires C, 54·1; H, 6·3; N, 14·8%).

5-(m-*Di*-2-*chloroethylaminophenyl*)*hydantoin*.—Vacuum-dried *m*-dihydroxyethylaminophenylhydantoin (13·2 g.) was dusted into phosphoryl chloride (100 ml.), and the mixture was stirred at 60°. Within 2 hr. a gum had been formed but after 7 hr. dissolution was complete. Next day the phosphoryl chloride was removed at 60°/30 mm.; dry benzene was then added and similarly removed. The residual brown gum was dissolved in concentrated hydrochloric acid (100 ml.), and the solution was evaporated at 60°/10 mm. An acetone solution of the residue was shaken with anhydrous sodium sulphate and then passed through a column of activated alumina containing charcoal. Addition of light petroleum (b. p. 40—60°) to the early eluates gave 5-(m-*di*-2-*chloroethylaminophenyl*)*hydantoin* as plates, m. p. 168° (Found: C, 49·5; H, 4·9; N, 13·3. $C_{13}H_{15}Cl_2N_3O_2$ requires C, 49·4; H, 4·8; N, 13·3%). The extent of hydrolysis under the standard conditions described in Part I [10] was 10·3%.

α-(m-*Di*-2-*chloroethylaminophenyl*)*glycine*.—The above hydantoin (3·3 g.) and concentrated hydrochloric acid (30 ml.) were heated in a sealed tube at 160—170° for 2 hr. When the solution was warmed on a steam-bath in a stream of air solid began to separate; at this point the addition of an excess of saturated aqueous sodium acetate caused the formation of a light buff precipitate which was collected and dried on a porous tile. When ether was added to a methanolic extract of this solid the *amino-acid* (1·1 g.) was obtained as plates, m. p. 179° (Found: C, 49·5; H, 5·7; N, 9·6. $C_{12}H_{16}Cl_2N_2O_2$ requires C, 49·5; H, 5·5; N, 9·6%).

3-*Methyl*-5-(m-*di*-2-*chloroethylaminophenyl*)*hydantoin*.—A suspension of the hydantoin (500 mg.) in ether (80 ml.) containing an excess of diazomethane (prepared from 2 g. of nitrosomethylurea) was stirred for 16 hr. No obvious reaction occurred and so acetone (20 ml.) was added and a clear solution was soon formed. Next day glacial acetic acid was added to destroy the excess of diazomethane, and the solvents were removed under reduced pressure. The N-*methyl derivative* was obtained as an amorphous powder, m. p. 142—144°, when light petroleum (b. p. 40—60°) was added to an acetone solution (Found: C, 51·0; H, 5·5; N, 12·8. $C_{14}H_{17}Cl_2N_3O_2$ requires C, 50·9; H, 5·2; N, 12·7%). The extent of hydrolysis under standard conditions was 10·9%.

5-p-*Aminophenylhydantoin*.—*p*-Aminobenzaldehyde [16] (72 g.), sodium cyanide (60 g.), and ammonium carbonate (450 g.) in 1 : 1 (v/v) aqueous ethanol (1800 ml.) were stirred at 55—60° for 10 hr. The crystals that began to separate during the reaction were filtered off from the cooled solution and washed with water. Concentration of the filtrate afforded a further quantity of product (total, 75 g.; m. p. 225—235°). On crystallisation from aqueous dimethylformamide the *hydantoin* (60 g.) formed pale yellow plates, m. p. 240—244° (decomp.). Repeated crystallisation from water (charcoal) gave colourless plates, m. p. 243—245° (decomp. with pre-sintering) (Found: C, 56·6; H, 4·6; N, 21·8. $C_9H_9N_3O_2$ requires C, 56·5; H, 4·8; N, 22·0%). The *acetyl derivative* formed plates, m. p. 303—305° (decomp.), from water (Found: C, 57·0; H, 4·9. $C_{11}H_{11}N_3O_3$ requires C, 56·7; H, 4·8%).

5-(p-*Di*-2-*hydroxyethylaminophenyl*)*hydantoin*.—Ethylene oxide (40 ml.) was added to a suspension of 5-*p*-aminophenylhydantoin (10 g.) in 1 : 1 aqueous acetic acid (100 ml.), and the mixture was stirred at room temperature for 16 hr. The solution was then concentrated and evaporated several times after the addition of benzene and ethanol, giving a thick syrup which eventually solidified (yield, 11·5 g.). Crystallisation from water gave the *dihydroxyethyl derivative* as plates, m. p. 229—231° (Found: C, 52·6; H, 6·0; N, 14·5. $C_{13}H_{17}N_3O_4,H_2O$ requires C, 52·5; H, 6·4; N, 14·1%).

5-(p-*Di*-2-*chloroethylaminophenyl*)*hydantoin*.—The dihydroxyethyl compound (4·5 g.) was stirred with phosphoryl chloride (30 ml.) at 60° for 16 hr., giving a clear reddish-brown solution. After evaporation under reduced pressure concentrated hydrochloric acid (10 ml.) was cautiously added. When this solution was poured into concentrated aqueous sodium acetate a grey-green solid separated. This was purified by passing an acetone solution through a column of activated alumina which had a layer of charcoal on top. Continued elution with acetone gave 5-(p-*di*-2-*chloroethylaminophenyl*)*hydantoin*, m. p. 155—156° [from acetone–light petroleum (b. p. 60—80°)] (Found: C, 49·2; H, 4·8; N, 13·0. $C_{13}H_{15}Cl_2N_3O_2$ requires C, 49·4; H, 4·8; N, 13·3%).

5-*Methyl*-5-m-*nitrophenylhydantoin*.—*m*-Nitroacetophenone (20 g.), ammonium chloride (25 g.), ammonium carbonate (40 g.), sodium cyanide (25 g.), methanol (200 ml.), and water

[16] Beard, Hodgson, and Davies, *J.*, 1944, 4.

(150 ml.) were heated in a pressure bottle at 60—70° for 26 hr. The suspension was then concentrated and acidified to Congo Red with hydrochloric acid. A brown solid (25·3 g.), m. p. 185—193°, was obtained. An ethanolic extract of the solid was evaporated and a solution of the product in acetone was passed through activated alumina. The eluates contained 5-*methyl*-5-m-*nitrophenylhydantoin*, pale yellow prisms, m. p. 195—196° (from water) (Found, for a specimen dried at 120°/0·001 mm.: N, 17·8. $C_{10}H_9N_3O_4$ requires N, 17·9%).

5-m-*Aminophenyl*-5-*methylhydantoin*.—5-Methyl-5-*m*-nitrophenylhydantoin (5 g.) in methanol (75 ml.) containing 5% palladium–charcoal (200 mg.) was shaken in hydrogen. The filtered solution was evaporated, giving a light yellow oil which solidified in contact with water. The *amine* formed pale yellow needles (3·6 g.), m. p. 77°, from water (Found, for a specimen dried at 40°/3 mm.: C, 53·9; H, 6·1; N, 18·5. $C_{10}H_{11}N_3O_2,H_2O$ requires C, 53·8; H, 5·9; N, 18·8%). The *acetyl derivative*, obtained by the action of acetic anhydride on an aqueous solution, formed yellow cubes, m. p. 247—248°, from water (Found: C, 58·3; H, 5·5; N, 16·7. $C_{12}H_{13}N_3O_3$ requires C, 58·3; H, 5·3; N, 17·0%).

5-(m-*Di*-2-*hydroxyethylaminophenyl*)-5-*methylhydantoin*.—The aminophenylhydantoin (6·95 g.), ethylene oxide (27·3 ml.), acetic acid (3·4 ml.), and water (50 ml.) were stirred at room temperature for 22 hr. Evaporation under reduced pressure gave a brown oil which redissolved in water to give a yellow solution. After treatment of this solution with charcoal, filtration, and concentration 5-(m-*di*-2-*hydroxyethylaminophenyl*)-5-*methylhydantoin* (3·8 g.) was obtained as prisms, m. p. 144—145° (Found, for a specimen dried at 135°/0·001 mm.: C, 57·7; H, 6·7; N, 14·1. $C_{14}H_{19}N_3O_4$ requires C, 57·3; H, 6·5; N, 14·3%).

5-(m-*Di*-2-*chloroethylaminophenyl*)-5-*methylhydantoin*.—Dried di-(2-hydroxyethyl)aminophenylhydantoin (12·56 g.) was added slowly to phosphoryl chloride (120 ml.), and the mixture was stirred for 2 hr. at 55°. Next day the phosphoryl chloride was removed under reduced pressure and the residue heated with hydrochloric acid. The solid which separated on addition of water to the concentrated acid solution was rapidly extracted with ether and after addition of solid sodium acetate to the aqueous layer this was again extracted. The combined ethereal extracts were dried (Na_2SO_4) and concentrated. When pentane was added until a faint turbidity formed and the solution was kept at 0°, needles (7·7 g.) of 5-(m-*di*-2-*chloroethylaminophenyl*)-5-*methylhydantoin*, m. p. 176—177°, separated (Found: C, 50·6; H, 5·1; N, 12·9. $C_{14}H_{17}Cl_2N_3O_2$ requires C, 50·9; H, 5·2; N, 12·7%). The extent of hydrolysis under standard conditions was 13·4%.

5-(m-*Di*-2-*chloroethylaminophenyl*)-3,5-*dimethylhydantoin*.—This was prepared by the action of ethereal diazomethane on the 5-monomethyl compound as described above, except that it was not necessary to add acetone. The *dimethyl derivative* formed needles, m. p. 154—155°, from acetone–pentane (Found: C, 51·9; H, 5·1; N, 11·8. $C_{15}H_{19}Cl_2N_3O_2$ requires C, 52·3; H, 5·6; N, 12·2%). The extent of hydrolysis under standard conditions was 10·2%.

α-(m-*Di*-2-*chloroethylaminophenyl*)*alanine*.—5-(*m*-Di-2-chloroethylaminophenyl)-5-methylhydantoin (3·4 g.) in concentrated hydrochloric acid (30 ml.) was heated in a sealed tube at 160—170° for 2 hr. Adding saturated sodium acetate to the concentrated and ice-cooled solution precipitated a solid; this was collected and dried on porous tile. The *amino-acid* was sparingly soluble in methanol, ethanol, acetone and ether but dissolved in hot acetic acid. It crystallised as prisms, m. p. 225° (gassing), R_F 0·80 in solvent *A*, when aqueous sodium acetate was added to a solution in dilute hydrochloric acid (Found, for a specimen dried at 100°/0·1 mm. for 4 hr.: C, 51·0; H, 5·7; N, 9·2. $C_{13}H_{18}Cl_2N_2O_2$ requires C, 51·1; H, 5·9; N, 9·2%).

5-*Methyl*-5-p-*nitrophenylhydantoin*.—*p*-Nitroacetophenone (33 g., 0·2 mole), sodium cyanide (20 g., 0·5 mole), and ammonium carbonate (145 g., 1·5 mole) in 1 : 1 (v/v) aqueous ethanol (600 ml.) were stirred at 55—60° for 8 hr. Crystalline hydantoin (32 g.), m. p. 225—227°, was filtered off and washed with water; a second crop (11 g.), m. p. 224—228°, was obtained on concentrating the mother-liquors to half bulk. 5-*Methyl*-5-p-*nitrophenylhydantoin* formed pale yellow prisms, m. p. 227—229°, from ethanol (Found: C, 50·8; H, 4·2; N, 17·7. $C_{10}H_9N_3O_4$ requires C, 51·1; H, 3·9; N, 17·9%).

5-p-*Aminophenyl*-5-*methylhydantoin*.—The nitrophenylhydantoin (23 g.) in dimethylformamide (150 ml.) was hydrogenated over 5% palladium–charcoal (2 g.). The uptake of hydrogen was slow, slightly more (6·8 l.) than the theoretical volume being absorbed in 8 hr. Evaporation of the filtered solution under reduced pressure gave a red oil which slowly solidified (13·8 g., 74%). Crystallisation from water (charcoal) gave the *aminophenylhydantoin* as prisms, m. p. 182—184° (Found: C, 58·6; H, 5·8; N, 20·0. $C_{10}H_{11}N_3O_2$ requires C, 58·5; H, 5·4;

N, 20·5%). This hydantoin, which was also obtained, in 51% yield, directly from *p*-aminoacetophenone, formed an *acetyl derivative*, plates, m. p. 261—263° (from aqueous methanol) (Found: C, 58·8; H, 5·6; N, 17·2. $C_{12}H_{13}N_3O_3$ requires C, 58·3; H, 5·3; N, 17·0%).

5-(p-*Di*-2-*hydroxyethylaminophenyl*)-5-*methylhydantoin*.—Ethylene oxide (15 ml.) was added to a solution of the aminophenylhydantoin (10 g.) in 1 : 1 (v/v) aqueous acetic acid (150 ml.), and the mixture was stirred at room temperature for 18 hr. The gum obtained after removal of the solvents under reduced pressure slowly solidified and recrystallised from water as a hydrate which lost solvent on drying (yield of dried product, 12 g.; m. p. 155—160°). The anhydrous *form* separated from acetone in rosettes, m. p. 161° (Found: C, 57·3; H, 6·3; N, 14·2. $C_{14}H_{19}N_3O_4$ requires C, 57·3; H, 6·5; N, 14·3%).

5-(p-*Di*-2-*chloroethylaminophenyl*)-5-*methylhydantoin*.—The hydroxyethyl compound (5 g.) was stirred with phosphoryl chloride (40 ml.) at 60° until dissolution was complete (2½ hr.). Next day the excess of phosphoryl chloride was removed under reduced pressure and the residue was heated with concentrated hydrochloric acid (20 ml.). When this solution was poured into saturated aqueous sodium acetate a green amorphous solid (4·9 g.) separated. This was purified by passage in acetone through activated alumina containing charcoal. Elution of the column with fresh acetone afforded the *aminophenyl-5-methylhydantoin* (3·3 g.) which formed prisms, m. p. 218—219°, from acetone (Found: C, 50·9; H, 5·4; N, 12·9. $C_{14}H_{17}Cl_2N_3O_2$ requires C, 50·9; H, 5·2; N, 12·7%). The rate of hydrolysis under standard conditions was 6·5%.

Condensation of p-*Nitrobenzaldehyde with Hydantoin*.—Hydantoin (4 g.), *p*-nitrobenzaldehyde (6 g.), and fused sodium acetate (6 g.) in glacial acetic acid (10 ml.) were heated over a free flame. In a short time the mass became solid and the product was collected and then washed with methanol and water. Crystallisation from acetic acid or water gave orange plates, m. p. >300°. This solid appears to be a *polymer* derived from the nitrobenzylidenehydantoin [Found: C, 51·3; H, 3·5; N, 17·5. $(C_{10}H_7N_3O_4)_x$ requires C, 51·5; H, 3·0; N, 18·0%] since material of m. p. 254° (Wheeler and Hofmann [5] and Johnson and Brautlecht [6] gave m. p. 254°) was eventually obtained when the above mixture was heated at 100° in a glycerol bath until a clear solution was obtained and then the temperature was raised to 160°; the nitrobenzylidenehydantoin soon separated. It was collected and washed with methanol for use in the next stage.

Reduction of 5-4′-*Nitrobenzylidenehydantoin*.—(*a*) A solution of 4-nitrobenzylidenehydantoin (5 g.) in dimethylformamide (50 ml.) containing 5% palladium–charcoal (400 mg.) was shaken in hydrogen. The product, when crystallised from aqueous ethanol, gave 5-4′-*aminobenzylidenehydantoin monohydrate* as plates, m. p. 192—194° (Found: C, 54·8; H, 5·1. $C_{10}H_9N_3O_2,H_2O$ requires C, 54·3; H, 5·0%). The aminobenzylidene compound was also obtained by using Adams platinum catalyst in acetic acid or Raney nickel catalyst in alkaline ethanolic solution.

(*b*) 5-4′-Nitrobenzylidenehydantoin (2 g.), red phosphorus (400 mg.), and concentrated hydrochloric acid (15 ml.; *d* 1·7) were heated under reflux for 2 hr. After concentration under reduced pressure and passage of sulphur dioxide the solution was neutralised with sodium hydroxide and evaporated to dryness. An ethanolic extract of the residue was treated with charcoal and evaporated. The residue, when crystallised from water, yielded 5-4′-aminobenzylhydantoin, prisms, m. p. 167°.

Alternative Preparation of 5-4′-*Aminobenzylhydantoin*.—*p*-Nitrophenylalanine [17] (12·7 g.) was dissolved in the minimum amount of boiling water, and potassium cyanate (5·2 g.) was added. After 30 minutes' heating on a steam-bath the mixture was neutralised with concentrated hydrochloric acid. A solution of the precipitated hydantoic acid (12 g.; m. p. 193—195°) in 20% aqueous hydrochloric acid (120 ml.) was heated on a steam-bath, 5-4′-nitrobenzylhydantoin soon crystallising [10·5 g.; m. p. 255° (slow heating), 240—244° (rapid heating) (Bucherer and Lieb [8] give m. p. 240—245°)]. Hydrogenation of the nitrobenzylhydantoin (5·35 g.) in dimethylformamide (100 ml.) over 5% palladium–charcoal (200 mg.) gave a small amount of a sparingly soluble green solid of high m. p. but the main product obtained by crystallising the material from water (charcoal) was 5-4′-aminobenzylhydantoin, m. p. 166—168°, identical with the compound described above (Found: for an air-dry specimen: C, 57·3; H, 5·4; N, 19·7; for a specimen dried at 100°/2 mm.: C, 58·6; H, 5·4; N, 20·5. Calc. for $C_{10}H_{11}N_3O_2$: C, 58·5; H, 5·4; N, 20·5%). Johnson and Brautlecht [6] give m. p. 145° and describe a hydrochloride, m. p. 255—257° (decomp.); our material gives a hydrochloride, m. p. 260—265° (decomp.) and an *acetyl derivative*, m. p. 254°, plates from water (Found: C, 58·0; H, 5·2; N, 16·8. $C_{12}H_{13}N_3O_3$ requires C, 58·3; H, 5·3; N, 17·0%).

[17] Erlenmeyer and Lipp, *Annalen*, 1883, **219**, 213.

5-(4-*Di*-2′-*hydroxyethylaminobenzyl*)*hydantoin*.—5-4′-Aminobenzylhydantoin (5 g.), ethylene oxide (10 ml.), and N-acetic acid (25 ml.) were stirred at room temperature for 16 hr. The mixture was then evaporated to dryness under reduced pressure and the residue was extracted with a large volume of hot acetone. On concentration, the acetone extract gave 5-(4-*di*-2′-*hydroxyethylaminobenzyl*)*hydantoin* (3·8 g.) as plates, m. p. 162—164° depressed to 140° by admixture with the aminobenzylhydantoin of m. p. 166° (Found, for a specimen dried at 100°/0·5 mm. for 6 hr.: C, 57·7; H, 6·8; N, 14·3. $C_{14}H_{19}N_3O_4$ requires C, 57·3; H, 6·5; N, 14·3%). In one run an acetone-insoluble residue was obtained which gave prisms, m. p. 187—189°, from aqueous acetone. This was apparently a *tri*-2-*hydroxyethyl derivative* (Found: C, 56·8; H, 7·0; N, 12·4. $C_{16}H_{23}N_3O_5$ requires C, 56·9; H, 6·9; N, 12·5%).

5-(4-*Di*-2′-*chloroethylaminobenzyl*)*hydantoin*.—The dihydroxyethylamino-derivative (3 g.) was added slowly to vigorously stirred phosphoryl chloride (30 ml.), and the mixture was kept at 50—60° for 2 hr. After removal of the excess of phosphoryl chloride at 60° under reduced pressure the purple gum which remained was dissolved in concentrated hydrochloric acid (30 ml.). This solution was concentrated to half-bulk at 60° and then covered with ether. An excess of aqueous sodium acetate was then added to the vigorously stirred mixture. The aqueous layer was extracted with more ether, and the combined extracts were washed with saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), and evaporated. A solution of the brown solid (2·2 g.) in dry acetone was passed through activated alumina containing charcoal which was then washed with fresh acetone. The eluates contained a white solid (1·2 g.) which could be purified by precipitation from acetone by pentane. 5-(4-*Di*-2′-*chloroethylaminobenzyl*)-*hydantoin* was obtained as an amorphous powder, m. p. 173—174° depressed to 142° by admixture with the hydroxyethylamino-compound of m. p. 164° (Found: C, 51·0; H, 5·2; N, 12·4. $C_{14}H_{17}Cl_2N_3O_2$ requires C, 50·9; H, 5·2; N, 12·7%). The extent of hydrolysis under standard conditions was 20·5%.

The N-*methyl derivative*, formed in the usual manner by ethereal diazomethane, was obtained as plates, m. p. 199—203°, from acetone–pentane (Found: C, 52·1; H, 5·9; N, 12·2. $C_{15}H_{19}Cl_2N_3O_2$ requires C, 52·3; H, 5·6; N, 12·2%). The extent of hydrolysis under standard conditions was 14·8%.

Hydrolysis of 5-(4-*Di*-2′-*chloroethylaminobenzyl*)*hydantoin*.—A solution of the hydantoin (500 mg.) in concentrated hydrochloric acid (5 ml.) was heated in a sealed tube at 160—170° for 2 hr. and then evaporated under reduced pressure. The residue was dissolved in ethanol saturated with dry hydrogen chloride and next day the solvent was removed. The product formed prisms, m. p. 160°, from chloroform–ethanol. This m. p. was not depressed by admixture with the authentic hydrochloride of the ethyl ester of *p*-di-2-chloroethylamino-DL-phenylalanine,[18] m. p. 159°, kindly supplied by Dr. J. A. Stock.

5-*Methyl*-5-4′-*nitrobenzylhydantoin*.—The enol acetate of 4-nitrobenzylacetone was prepared by Smith's method [19] and hydrolysed by refluxing for 1 hr. in concentrated hydrochloric acid (1 vol.) and ethanol (10 vol.), giving the ketone, m. p. 59—61° (Overberger and Biletch [20] record m. p. 62—63°). 4-Nitrobenzylacetone (36 g.), sodium cyanide (20 g.), and ammonium carbonate (150 g.) in 50% aqueous ethanol (600 ml.) were stirred at 55—60° for 10 hr. The filtered solution was then concentrated under reduced pressure and the crude brown hydantoin (46 g.) was collected by filtration. Crystallisation from ethyl acetate and then from aqueous acetone gave pale yellow needles of 5-*methyl*-5-4′-*nitrobenzylhydantoin* (25·8 g.), m. p. 217—219° with presintering (Found: C, 53·2; H, 4·2; N, 17·0. $C_{11}H_{11}N_3O_4$ requires C, 53·0; H, 4·5; N, 16·9%).

5-4′-*Aminobenzyl*-5-*methylhydantoin*.—The nitrobenzylhydantoin (27·5 g.) in dimethylformamide (150 ml.) was hydrogenated over 5% palladium–charcoal (3 g.); the theoretical volume of gas (7·5 l.) was taken up during 1½ hr. The syrup (17·4 g.) obtained by evaporating the filtered solution solidified in contact with ethyl acetate and on crystallisation from this solvent 5-4′-*aminobenzyl*-5-*methylhydantoin* formed rosettes, m. p. 187—189° (Found: C, 60·1; H, 5·9; N, 19·3. $C_{11}H_{13}N_3O_2$ requires C, 60·3; H, 6·0; N, 19·2%). It afforded an *acetyl derivative*, plates, m. p. 300° (decomp.) with sintering at 280°, from ethanol (Found: C, 59·8; H, 5·8; N, 15·6. $C_{13}H_{15}N_3O_3$ requires C, 59·8; H, 5·8; N, 16·1%).

[18] Vodolazskaja, Novikova, Shkodinskaja, Berlin, and Larionov, *Oncologia*, 1957, **44** (No. 11), 76; J. A. Stock, personal communication.
[19] Smith, *J. Amer. Chem. Soc.*, 1953, **75**, 1134.
[20] Overberger and Biletch, *J. Amer. Chem. Soc.*, 1951, **73**, 4880.

5-(4-*Di*-2′-*chloroethylaminobenzyl*)-5-*methylhydantoin*.—The aminobenzylhydantoin (5·6 g.), ethylene oxide (12·7 ml.), acetic acid (1·2 ml.), and water (18·8 ml.) were stirred at room temperature overnight and then evaporated to dryness, giving a gum (8 g.). This material (5·1 g.) was stirred at 25° in phosphoryl chloride (50 ml.). After 16 hr. solution was complete and the excess of reagent was removed at 80° under reduced pressure. Dry benzene was added and removed in vacuum and the product was heated for ½ hr. with concentrated hydrochloric acid (20 ml.). The concentrated solution was covered with ether and diluted with water with vigorous stirring. Addition of light petroleum (b. p. 40—60°) to the dried ether solution gave the *monohydrate* of 5-(4-di-2′-chloroethylaminobenzyl)-5-methylhydantoin as needles, m. p. 95—97° (2 g.) (Found: C, 49·9; H, 5·8; N, 11·8; Cl, 19·6. $C_{15}H_{19}Cl_2N_3O_2,H_2O$ requires C, 49·7; H, 5·8; N, 11·6; Cl, 19·6%). The extent of hydrolysis under standard conditions was 19%.

5-4′-*Aminophenethyl*-5-*methylhydantoin*.—A 1% solution of sodium hydroxide (52·5 ml.) was added dropwise to a stirred mixture of *p*-nitrobenzaldehyde (63 g.) and acetone (550 ml.) kept at −5°. The mixture was kept at room temperature for 4 hr. and then neutralised with 2N-hydrochloric acid. The dark oil obtained when the acetone was removed under reduced pressure slowly solidified and after being washed with water and dried it crystallised from ether as pale yellow prisms (40 g.), m. p. 58—60° (Combes *et al.*[21] give m. p. 60—61°). The ketol (50 g.) was dehydrated in acetic anhydride (200 ml.) on a steam-bath (1½ hr.). On dilution with water (250 ml.) 4-nitrobenzylideneacetone separated; it formed yellow needles (42 g.), m. p. 105—108°, from ethanol. The nitro-compound (26·5 g.) in ethyl acetate (200 ml.) was hydrogenated over 5% palladium charcoal (1·5 g.), 3 mols. of hydrogen (12·0 l.) being taken up during 2 hr. 4-Aminophenethyl methyl ketone was obtained as an orange-yellow oil (22·5 g.) which was characterised as its N-*benzoyl derivative*, plates, m. p. 131° (from aqueous methanol) (Found: C, 76·5; H, 6·7; N, 5·2. $C_{17}H_{17}NO_2$ requires C, 76·4; H, 6·4; N, 5·2%). The oily aminoketone (22 g.), sodium cyanide (13 g.), and ammonium carbonate (90 g.) in 50% aqueous ethanol (400 ml.) were heated at 55—60° for 8 hr. 5-4′-*Aminophenethyl*-5-*methylhydantoin* (19 g.), isolated in the usual manner, formed needles, m. p. 208—209°, from methanol (Found: C, 61·4; H, 6·7; N, 18·0. $C_{12}H_{15}N_3O_2$ requires C, 61·8; H, 6·5; N, 18·3%). The *acetyl derivative* was obtained as plates, m. p. 244—246°, from aqueous methanol (Found: C, 60·9; H, 6·5; N, 15·3. $C_{14}H_{17}N_3O_3$ requires C, 61·1; H, 6·2; N, 15·3%).

5-(4-*Di*-2′-*hydroxyethylaminophenethyl*)-5-*methylhydantoin*.—The above amine (25 g.) and ethylene oxide (50 ml.) in 50% (v/v) aqueous acetic acid (300 ml.) were stirred at 20° for 16 hr. The residue obtained after removal of the solvent under reduced pressure crystallised on the addition of light petroleum (b. p. 40—60°) to an acetone solution. Colourless needles (19·1 g.), m. p. 135—136°, of the *bishydroxyethyl derivative* were thus obtained (Found: C, 60·0; H, 7·1; N, 12·6. $C_{16}H_{23}N_3O_4$ requires C, 59·8; H, 7·2; N, 13·1%).

5-(4′-*Di*-2-*chloroethylaminophenethyl*)-5-*methylhydantoin*.—A suspension of the dihydroxyethyl compound (10 g.) in phosphoryl chloride (80 ml.) was stirred at 55—60° for 1 hr. and then at room temperature for a further 16 hr. After removal of the excess of reagent under reduced pressure the product was warmed for 1 hr. with concentrated hydrochloric acid (15 ml.), then poured into cold saturated aqueous sodium acetate (400 ml.). The gum which separated was extracted with ether, washed with aqueous sodium hydrogen carbonate, and dried ($MgSO_4$). This extract contained 5-(4-*di*-2′-*chloroethylaminophenethyl*)-5-*methylhydantoin* (7·6 g.) which formed needles, m. p. 142—143°, from acetone–light petroleum (b. p. 40—60°) (Found: C, 53·9; H, 6·1; N, 11·9. $C_{16}H_{21}Cl_2N_3O_2$ requires C, 53·7; H, 5·9; N, 11·7%). The rate of hydrolysis under standard conditions was 24%.

5-*Methyl*-5-(3-p-*nitrophenylpropyl*)*hydantoin*.—Methyl 3-*p*-nitrophenylpropyl ketone[22] (52 g.), sodium cyanide (30 g.), and ammonium carbonate (250 g.) in 1 : 1 aqueous ethanol (1 l.) were stirred at 55—60° for 10 hr. On cooling, the *hydantoin* (56 g.) separated; it was purified by extracting its solution in 2N-sodium hydroxide with ether and precipitating the product with 2N-hydrochloric acid. Recrystallisation from glacial acetic acid gave prisms, m. p. 135—140° (26·5 g.) (Found: C, 56·0; H, 5·5. $C_{13}H_{15}N_3O_4$ requires C, 56·3; H, 5·5%).

5-(3-*Aminophenylpropyl*)-5-*methylhydantoin*.—The nitro-compound (26·5 g.) in ethyl acetate (600 ml.) was reduced over 5% palladium–charcoal (2·5 g.); uptake of hydrogen was complete in ½ hr. Evaporation of the filtered solution afforded prisms, m. p. 146—147°, of the

[21] Combes, Hebbelynck, and Ledrut, *Bull. Soc. chim. France*, 1953, 315.
[22] Dale and Strobel, *J. Amer. Chem. Soc.*, 1954, **76**, 6172.

aminohydantoin (14 g.) (Found: C, 63·1; H, 6·9; N, 17·0. $C_{13}H_{17}N_3O_2$ requires C, 63·1; H, 6·9; N, 17·0%). The *acetyl derivative* formed plates, m. p. 199°, from water (Found: C, 62·1; H, 6·5; N, 14·0. $C_{15}H_{19}N_3O_3$ requires C, 62·3; H, 6·6; N, 14·5%).

5-(3-p-*Di*-2′-*hydroxyethylaminophenylpropyl*)-5-*methylhydantoin*.—A solution of the aminohydantoin (12 g.) in 1 : 1 (v/v) aqueous acetic acid (150 ml.) containing ethylene oxide (25 ml.) was kept at room temperature for 16 hr. Evaporation of the solvent under reduced pressure gave a gum which became solid (16·7 g.) when rubbed with aqueous sodium hydrogen carbonate. The *di*-2-*hydroxyethyl derivative* formed rosettes, m. p. 125—135°, from water (Found: C, 60·5; H, 7·2; N, 12·4. $C_{17}H_{25}N_3O_4$ requires C, 60·9; H, 7·5; N, 12·5%).

5-(3-*Di*-2′-*chloroethylaminophenylpropyl*)-5-*methylhydantoin*.—The hydroxyethyl derivative (10 g.) was stirred with phosphoryl chloride (80 ml.) at 70° for 1 hr. and then left at room temperature for 2 hr. Removal of the excess of reagent left a gum which was warmed with concentrated hydrochloric acid (15 ml.) on a steam-bath for ½ hr. The resinous precipitate formed when the cooled solution was poured into 10% aqueous sodium acetate (300 ml.) was immediately extracted with ether, and the extracts were thoroughly washed with aqueous sodium hydrogen carbonate and dried ($MgSO_4$). The *di*-2-*chloroethyl derivative* (7·1 g.) obtained on evaporation formed prisms, m. p. 158—160°, from acetone–light petroleum (b. p. 40—60°) (Found: C, 55·3; H, 6·3; N, 11·0. $C_{17}H_{23}Cl_2N_3O_2$ requires C, 54·9; H, 6·2; N, 11·3%). The extent of hydrolysis under standard conditions was 31%.

Hydantoin-5-*spiro*-1′-(7′-*amino*-1′,2′,3′,4′-*tetrahydronaphthalene*).—7-Amino-1-tetralone [23] (26 g.), sodium cyanide (15 g.), and ammonium carbonate (135 g.) in 1 : 1 aqueous ethanol (500 ml.) were stirred at 60° for 8 hr. The *hydantoin* (77%) formed prisms, m. p. 251—252° (decomp.), from ethanol (Found: C, 62·1; H, 5·5; N, 17·7. $C_{12}H_{13}N_3O_2$ requires C, 62·3; H, 5·7; N, 18·2%).

Hydantoin-5-*spiro*-1′-(7′-*di*-2″-*hydroxyethylamino*-1′,2′,3′,4′-*tetrahydronaphthalene*).—A solution of the aminohydantoin (25 g.) in 1 : 1 aqueous acetic acid (300 ml.) containing ethylene oxide (50 ml.) was kept at room temperature for 16 hr. and then evaporated under reduced pressure. The *di*-2-*hydroxyethyl derivative* (24 g.) formed rosettes, m. p. 186—188°, from ethyl acetate (Found: C, 60·6; H, 6·6; N, 13·1. $C_{16}H_{21}N_3O_4$ requires C, 60·2; H, 6·6; N, 13·2%).

Hydantoin-5-*spiro*-1-(7′-*di*-2″-*chloroethylamino*-1′,2′,3′,4′-*tetrahydronaphthalene*).—The hydroxyethyl derivative (10 g.) was stirred with phosphoryl chloride (80 ml.) at 70° for 1½ hr. When dissolution was complete, the whole was heated with concentrated hydrochloric acid and then poured into sodium acetate solution and extracted as described above. Crystallisation from acetone–light petroleum (b. p. 60—80°) gave the *di*-2-*chloroethyl derivative* (7·4 g.) as needles m. p. 165—167° (Found: C, 54·4; H, 5·4; N, 12·1. $C_{16}H_{19}Cl_2N_3O_2$ requires C, 54·0; H, 5·4; N, 11·8%). The extent of hydrolysis under standard conditions was 14%.

1-*Amino*-7-*di*-2′-*chloroethylamino*-1,2,3,4-*tetrahydro*-1-*naphthoic Acid*.—The above hydantoin (5 g.) in concentrated hydrochloric acid (50 ml.) was heated in a sealed tube at 160—170° for 2 hr. On addition of saturated aqueous sodium acetate to the concentrated and cooled solution a gum separated and this solidified in contact with acetone. The *amino-acid* crystallised from a large volume of ethanol as needles of indefinite m. p. (shrinking at 165—175°, frothing at 210—220°, and finally liquefying at 230°), R_F in solvent *A* 0·82. This behaviour was unchanged on repeated crystallisation (Found, for a specimen dried at 100°/0·2 mm. for 2 hr.: C, 53·0; H, 6·4; N, 8·1. $C_{15}H_{20}Cl_2N_2O_2,\frac{1}{2}H_2O$ requires C, 53·0; H, 6·2; N, 8·2%).

Attempted Preparation of 5-o-*Aminophenylhydantoin*.—*o*-Aminobenzaldehyde (5·26 g.), sodium cyanide (4·3 g.), and ammonium carbonate (16·7 g.) in 1 : 1 aqueous ethanol (250 ml.) were heated and stirred in a pressure bottle at 60—70° for 16 hr. The *product* (4·7 g.) separated from the cooled solution. Crystallisation from a large volume of water gave pale yellow needles, m. p. 280—281° (decomp.) (Found: C, 56·2; H, 4·8; N, 22·2. $C_9H_9N_3O_2$ requires C, 56·5; H, 4·8; N, 22·0%), $\lambda_{max.}$ 250 and 286 mμ (log ε 3·85 and 3·27). Attempts to prepare an acetyl, benzoyl, or di-2-hydroxyethyl derivative were unsuccessful and an amino-group could not be detected by a diazotisation and coupling test. The chlorine-free residue left when the hydantoin was evaporated with concentrated hydrochloric acid formed plates, m. p. 239—240° from water (Found: C, 54·1, 54·2; H, 4·2, 4·7; N, 14·3, 14·6. Calc. for $C_9H_8N_2O_3$: C, 56·3; H, 4·2; N, 14·6%), $\lambda_{max.}$ 250 and 286 mμ (log ε 3·79 and 3·20). Harvill and Herbst [24] give m. p. 240—244° for 5-*o*-hydroxyphenylhydantoin.

[23] Vesely and Sturza, *Coll. Czech. Chem. Comm.*, 1933, **5**, 343.
[24] Harvill and Herbst, *J. Org. Chem.*, 1944, **9**, 21.

5-o-*Chlorophenylhydantoin.*—*o*-Chlorobenzaldehyde was converted into the hydantoin by the process described above. The contents of the pressure bottles were concentrated, acidified with hydrochloric acid, and evaporated to dryness. 5-*o*-Chlorophenylhydantoin [24,25] which was extracted from the residue with hot acetone, formed plates, m. p. 175—177°, on slow cooling of an aqueous solution (Found: C, 51·2; H, 3·3; N, 13·3; Cl, 16·8. Calc. for $C_9H_7ClN_2O_2$: C, 51·4; H, 3·4; N, 13·3; Cl, 16·8%); it showed no absorption maxima in the range 240—340 mμ. No identifiable product could be obtained by heating the chlorohydantoin with diethanolamine under a variety of conditions.

Diethyl α-Acetamido-2-nitrobenzylmalonate.—Diethyl acetamidomalonate (4·82 g.) was added to a solution of sodium (0·9 g.) in dry ethanol (600 ml.). An immediate turbidity formed on addition of 2-nitrobenzyl chloride [26] (4·35 g.) to this solution and reaction was completed under reflux in 3 hr. Removal of the alcohol under reduced pressure gave an oil which was shaken with ether and water. The dried ethereal layer deposited needles (2 g.), m. p. 106°, of *diethyl α-acetamido-2-nitrobenzylmalonate* when light petroleum (b. p. 40—60°) was slowly added (Found: C, 54·2; H, 5·9; N, 7·9. $C_{16}H_{20}N_2O_7$ requires C, 54·5; H, 5·7; N, 7·95%).

Reduction of Diethyl Acetamido-2-nitrobenzylmalonate.—The diester was hydrogenated in ethanol solution over 5% palladium–charcoal. Concentration of the filtered solution gave an oil which solidified in contact with ether. The *lactam* formed needles or prisms (depending on the rate of cooling), m. p. 190—191°, from water (Found: C, 61·1; H, 5·9; N, 10·2. $C_{14}H_{16}N_2O_4$ requires C, 60·9; H, 5·8; N, 10·1%).

o-*Nitrophenylalanine.*—Diethyl acetamido-2-nitrobenzylmalonate (39·7 g.) in concentrated hydrochloric acid (175 ml.) and water (175 ml.) was heated under reflux for 17 hr. Evaporation gave a residue which crystallised when dissolved in concentrated hydrochloric acid (charcoal) and cooled to 0°; yellow needles of the *hydrochloride*, m. p. 222—223° (decomp.), were obtained (24·8 g.) (Found: C, 44·2; H, 4·7; N, 11·6%; equiv., Volhard titration of Cl^-, 246 5. $C_9H_{10}N_2O_4$,HCl requires C, 43·9; H, 4·5; N, 11·4%; equiv., 246·7).

β-o-*Nitrophenyl-α-ureidopropionic acid.*—Potassium cyanate (12 g.) was added to *o*-nitro phenylalanine hydrochloride (11·47 g.) dissolved in the smallest quantity of water, and the mixture was heated on a steam-bath for ½ hr. Adjusting the pH of the solution to 4 caused separation of the *ureido-acid* (6·6 g.), pale yellow glistening plates, m. p. 203—204° (from water) (Found: C, 47·8; H, 4·6; N, 16·4. $C_{10}H_{11}N_3O_5$ requires C, 47·4; H, 4·4; N, 16·6%).

5-2′-*Nitrobenzylhydantoin.*—A solution of the ureido-acid (6·6 g.) in concentrated hydrochloric acid (80 ml.) and water (80 ml.) was heated on a steam-bath for ½ hr. The solid (5·75 g.) which separated formed yellow needles, m. p. 238° (decomp.), from aqueous ethanol (Found: C, 50·7; H, 3·6; N, 17·4. Calc. for $C_{10}H_9N_3O_4$: C, 51·1; H, 3·9; N, 17·9%). Bucherer and Lieb [8] give m. p. 213—215°.

Reduction of 5-2′-*Nitrobenzylhydantoin.*—The nitrobenzylhydantoin (5 g.) was hydrogenated in dimethylformamide (200 ml.) as described above. The *product* formed needles, m. p. 303—304° (decomp. and very dependent on the rate of heating), from aqueous ethanol (Found: C, 64·3; H, 4·7; N, 22·1. $C_{10}H_9N_3O$ requires C, 64·2; H, 4·85; N, 22·45%). It was unchanged on treatment with acetic anhydride or with ethylene oxide in dilute acetic acid. It had a broad maximum at 280 mμ (log ε 4·06) and sharp maxima at 324 and 332 mμ (log ε 3·41 and 3·52). 5-Benzylhydantoin exhibits sharp maxima at 260 and 265 mμ (log ε 2·4 and 2·25).[27]

2-*Di*-2′-*hydroxyethylaminobenzyl Alcohol.*—2-Aminobenzyl alcohol [28] (28·1 g.) and ethylene oxide (22·5 ml.) in sodium-dried benzene (28 ml.) were heated at 170—175° for 5 hr. in a sealed tube. The product was distilled and two main fractions collected: (*a*) b. p. 186—188°/0·3 mm. (2 g.), and (*b*) 196—200°/0·05 mm. (36·6 g.). Fraction (*a*) deposited 2-2′-*hydroxyethylaminobenzyl alcohol*, m. p. 76—77°, needles from ether (Found: C, 64·5; H, 7·8; N, 8·0. $C_9H_{13}NO_2$ requires C, 64·65; H, 7·8; N, 8·4%). Fraction (*b*) was the required 2-*di*-2′-*hydroxyethylaminobenzyl alcohol* (Found: C, 62·6; H, 8·15; N, 6·7. $C_{11}H_{17}NO_3$ requires C, 62·5; H, 8·1; N, 6·6%) which did not crystallise but formed a *tri*-p-*nitrobenzoate*, m. p. 132—133°, pale yellow prisms from acetone (Found: C, 57·9; H, 4·2; N, 8·5. $C_{32}H_{26}N_4O_{12}$ requires C, 58·4; H, 4·05; N, 8·7%).

2-*Di*-2′-*chloroethylaminobenzyl Chloride.*—The triol (7·8 g.) was dissolved in warm benzene

[25] Henze and Speer, *J. Amer. Chem. Soc.*, 1942, **64**, 522.
[26] Gabriel and Borgmann, *Ber.*, 1883, **16**, 2064.
[27] McLean and Seeger, *J. Amer. Chem. Soc.*, 1940, **62**, 1416.
[28] Nystrom and Brown, *J. Amer. Chem. Soc.*, 1947, **69**, 2548.

(25 ml.), and phosphoryl chloride (12 ml.) was cautiously added. After being heated on a steam-bath for 12 hr. the whole was evaporated and a light petroleum (b. p. 40—60°) extract of the residue was passed through a short column of activated alumina. Distillation of the material in the eluates gave two main fractions, (*a*) b. p. 138—142°/0·01 mm. (1 g.), and (*b*) 142°/0·01 (3·4 g.). Fraction (*b*) was the required *trichloro-compound* (Found: C, 50·2; H, 5·4; N, 5·2; Cl, 37·1. $C_{11}H_{14}NCl_3$ requires C, 49·6; H, 5·3; N, 5·25; Cl, 39·9%).

Diethyl Acetamido-2-di-2′-chloroethylaminobenzylmalonate.—Carefully dried diethyl acetamidomalonate (11·6 g.) was added to a solution from sodium (1·23 g.) in anhydrous ethanol (150 ml.). After addition of the trichloro-compound (14·3 g.) in anhydrous ethanol (50 ml.) the mixture was stirred at room temperature. A turbidity developed within 20 min. but stirring was continued until the mixture no longer showed an alkaline reaction (about 22 hr.). Removal of the solvent in a flash-evaporator gave a residue which was extracted with ether. Adding light petroleum (b. p. 40—60°) to the dried extract precipitated an oil that slowly solidified. The low-melting product was dissolved in benzene and sufficient light petroleum was added to render the solution slightly turbid. Unchanged acetamidomalonate (2·7 g.) slowly separated. The mother-liquor was passed through activated alumina. Early eluates contained trichloro-compound (5 g.) but continued elution with benzene–light petroleum (b. p. 60—80°; 1 : 3) gave solids, m. p. 51—64°. These solid fractions were combined and crystallised from benzene–pentane, giving *diethyl acetamido-2-di-2′-chloroethylaminobenzylmalonate* (4·1 g.), m. p. 67—68°. Recrystallisation from a large volume of light petroleum (b. p. 60—80°) gave needles, m. p. 69—70° (Found: C, 53·6; H, 6·1; N, 6·3; Cl, 16·0. $C_{20}H_{28}Cl_2N_2O_5$ requires C, 53·7; H, 6·3; N, 6·3; Cl, 15·9%).

o-*Di-2-chloroethylamino*-DL-*phenylalanine.*—A solution of the diester (1·53 g.) in concentrated hydrochloric acid (15 ml.) was heated under reflux for 2½ hr. and then evaporated on a steam-bath to 5 ml. On addition of ice-cooled saturated aqueous sodium acetate at 0° a gum separated. The aqueous layer was poured into a separating funnel and the gum was dissolved in a little acetone and then benzene or chloroform (25 ml.) was added, and this solution was rapidly used to extract the aqueous layer. The organic layer was dried (Na_2SO_4) and pentane was gradually added, causing the precipitation of a granular solid—rapid addition causes the formation of an oil. The solid (850 mg.) was collected and washed with cold acetone and recrystallised by dissolving it in the minimum quantity of cold acetic acid and adding ether. The *amino-acid* very slowly separated as rosettes of needles; the behaviour on heating is characteristic: the specimen remains firm up to 162° and then collapses and begins to froth. When purified the amino-acid is moderately soluble in methanol and sparingly soluble in ethanol, acetone, and ether but very soluble in cold acetic acid (Found, for a specimen dried at 60°/0·2 mm. for 4 hr.: C, 51·0; H, 5·9; N, 9·0; Cl, 23·2%; equiv. by formol titration, 310. $C_{13}H_{18}Cl_2N_2O_2$ requires C, 51·2; H, 5·9; N, 9·2; Cl, 23·2%; equiv., 305); it had R_F in solvent *A* 0·79, in solvent *B* 0·64. Under the same conditions the *para*-isomer had R_F in solvent *A* 0·73, in solvent *B* 0·51; and the *meta*-isomer had R_F in solvent A 0·75, in solvent B 0·55. The rate coefficient for hydrolysis of *o*-di-2-chloroethylaminophenylalanine in water containing 1% of methanol at pH 7 and 37°, determined by Dr. Davis using a Radiometer automatic titrator, was $k = 3{\cdot}27 \times 10^{-4}$ sec.$^{-1}$ (half-life, 35·2 min.). Under similar conditions the rate coefficient for hydrolysis of the *para*-isomer was $k = 1{\cdot}45 \times 10^{-4}$ sec.$^{-1}$ (half-life, 79·7 min.).[14]

γ-p-(N-2-*Chloroethyl*-N-*ethylaminophenyl*)*butyric Acid.*—Methyl *p*-aminophenylbutyrate [7] (21 g.), Raney nickel (30 g.), and ethanol (100 ml.) were heated under reflux with stirring for 3 hr. Evaporation of the filtered solution gave the *N*-ethylamino-ester as a yellow oil (22·3 g.). This oil (18 g.) in 4N-acetic acid (120 ml.) and ethylene oxide (25 ml.) was stirred at room temperature for 16 hr. The product was isolated by evaporation and extraction with ether and then dissolved in benzene (150 ml.). Residual moisture was removed by distilling off 50 ml. of benzene and then phosphoryl chloride (15 ml.) was added and heating continued for 1 hr. The residue obtained by evaporation under reduced pressure was heated with concentrated hydrochloric acid (40 ml.) for 1½ hr. Dilution with water and addition of sodium acetate solution precipitated an oil which eventually solidified and was crystallised repeatedly from light petroleum (b. p. 60—80°). The *monochloroethyl derivative* (6·4 g.) formed plates, m. p. 61—63° (Found: C, 62·5; H, 7·5; N, 5·5. $C_{14}H_{20}ClNO_2$ requires C, 62·3; H, 7·5; N, 5·2%).

N-4-*Aminophenethylphthalimide.*—Potassium phthalimide (20 g.) was added during 10 min. to a stirred solution of 4-nitrophenethyl bromide (23 g.) in dimethylformamide (80 ml.). The mixture was heated at 110° for ¾ hr. and then at 80° for 3 hr., then poured into water. A solid

separated (34 g.) and was collected, dried, and crystallised from propan-1-ol as needles, m. p. 198—199° (Found: C, 64·9; H, 4·1. Calc. for $C_{16}H_{12}N_2O_4$: C, 64·9; H, 4·1%). Bergel *et al.*[29] report that the nitro-compound formed plates, m. p. 204—205°, from pentyl alcohol. The amine, m. p. 160°, was obtained by hydrogenation in ethyl acetate–methanol over palladium–charcoal (Bergel *et al.*[29] give m. p. 162°).

N-4-*Ethylaminophenethylphthalimide*.—The amine (10 g.), Raney nickel (15 g.), and ethanol (60 ml.) were heated under reflux for 3½ hr. Acetone was added to dissolve the solid which had separated and the solution was filtered and concentrated, giving the *ethylamino-compound* (8·0 g.), prisms, m. p. 151—152° (Found: C, 73·5; H, 6·2; N, 9·7. $C_{18}H_{18}N_2O_2$ requires C, 73·5; H, 6·2; N, 9·5%).

N-4-*Ethylhydroxyethylaminophenethylphthalimide*.—A solution of the ethylamine (16·5 g.) and ethylene oxide (25 ml.) in 50% acetic acid (200 ml.) was stirred for 15 hr. Evaporation gave the *hydroxyethyl derivative* (17·2 g.) which formed prisms, m. p. 117°, from chloroform–light petroleum (b. p. 60—80°) (Found: C, 70·7; H, 6·6; N, 8·7. $C_{20}H_{22}N_2O_3$ requires C, 71·0; H, 6·6; N, 8·3%).

N-*Chloroethyl*-N-*ethylaminophenethylamine Dihydrochloride*.—The dried hydroxyethyl derivative (17·0 g.), phosphoryl chloride (15 ml.), and benzene (120 ml.) were heated under reflux for 1 hr., and then the solvent and excess of reagent were removed under reduced pressure. The gummy residue was heated under reflux for 3 hr. with concentrated hydrochloric acid (150 ml.). After concentration to about 40 ml. under reduced pressure the cooled solution was basified with sodium carbonate and extracted with ether. The ether solution was extracted with dilute hydrochloric acid, and the aqueous layer was concentrated, giving hygroscopic needles, m. p. 200—206° (decomp.), of the dihydrochloride. Recrystallisation from methanol–ethyl acetate raised the m. p. to 205—208° (decomp.) (Found: C, 48·3; H, 7·4; N, 9·1. $C_{12}H_{21}Cl_3N_2$ requires C, 48·1; H, 7·1; N, 9·3%).

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The Chester Beatty Research Institute,
Institute of Cancer Research: Royal Cancer Hospital,
Fulham Road, London, S.W.3. [*Received, March 7th*, 1960.]

[29] Bergel, Everett, Roberts, and Ross, *J.*, 1955, 3835.