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ARYL-2-HALOGENOALKYLAMINES—XX*

THE PREPARATION AND PROPERTIES OF SOME DI-2-CHLOROETHYL-AMINOARYL SUBSTITUTED HYDANTOINS AND RELATED AMINO ACIDS

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Abstract—A number of di-2-chloroethylaminoaryl hydantoins and the amino acids into which they would be converted by hydrolysis have been examined for chemical reactivity, toxicity and effectiveness as inhibitors of the transplanted Walker rat carcinoma. It has been shown that in some cases potentiation of activity would be expected if a hydantoin were converted into an amino acid *in vivo*. The failure to observe a higher than average chemotherapeutic index with respect to the Walker tumour argues against selective hydrolysis of the hydantoin in the tissues of this tumour.

DERIVATIVES of aryl-2-halogenoalkylamines ("aromatic nitrogen mustards") in which the initially low reactivity of the halogen atoms can be increased by modification of the molecule *in vivo* are said to possess latent activity (see ref. 1 for a review of this concept). The more reactive and hence more cytotoxic alkylating agent will be released in those sites capable of effecting the required modification. In this way it should be possible to produce selective cytotoxic activity.

A number of di-2-chloroethylaminoaryl amino acids are very effective anti-tumour agents (especially the three isomeric phenylalanine derivatives) and it is known that hydantoins are metabolized to give amino acids.[2] The high activity of the amino acid derivatives is probably associated with favourable transport across cellular membranes and so *in vivo* hydrolysis of a hydantoin will generate locally a more effective agent. If, in addition, the amino acid possesses more reactive halogen atoms there should be and extra enhancement of biological activity. The preparation, chemical reactivity, and tumour growth inhibiting activity of a number of hydantoins and related amino acids are described in the present paper.

MATERIALS AND METHODS

The preparation of several of the hydantoins and amino acids investigated in the present work has already been described.[3] Two new indane derivatives and a tetralin derivative have now been prepared. 6-Aminoindan-1-one (I) was converted into the spirohydantoin (II) and this on successive treatment with ethylene oxide and thionyl chloride afforded hydantoin-5-spiro-1′-(6′-di-2″-chloroethylaminoindane) (III). Acid hydrolysis under pressure converted the hydantoin into the corresponding amino acid (IV).

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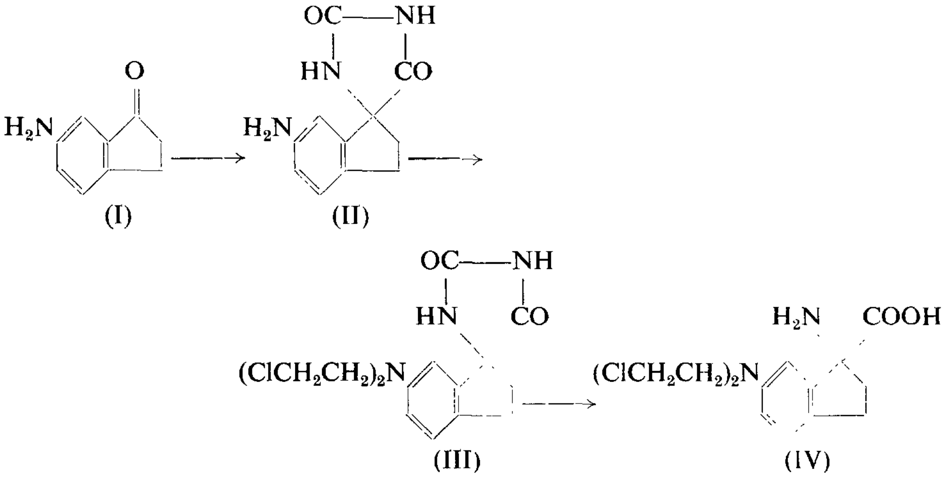
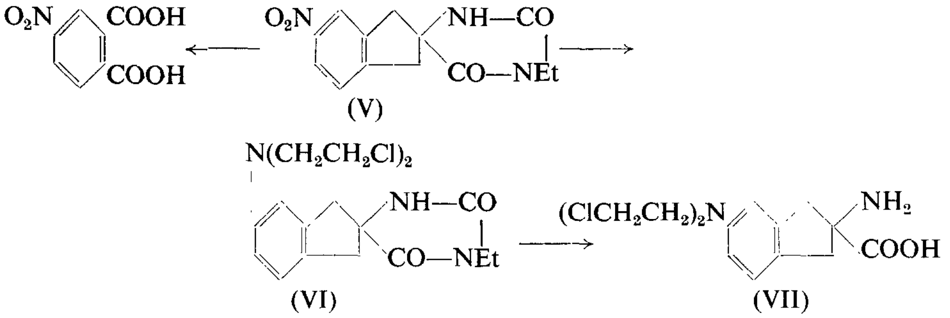
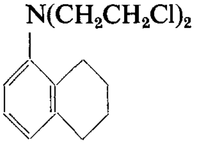


Nitration of the hydantoin derived from indan-2-one gave hydantoin-5-spiro-2′-(5′-nitroindane) which was most conveniently purified as its 3-ethyl derivative (V). The orientation of the nitro group in this derivative was established by the formation of 3-nitrophthalic acid on oxidation. The nitro compound was converted into the amino-, di-2-hydroxyethylamino-, and di-2-chloroethylamino- (VI), derivatives by the usual methods and acid hydrolysis then afforded the amino acid (VII)

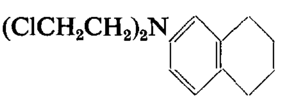


Similarly nitration of hydantoin-5-spiro-2′-(1′,2′,3′,4′-tetrahydronaphthalene) afforded a mixture of mononitro derivatives which was converted into a mixture of amino derivatives by catalytic reduction and then treated with ethylene oxide in aqueous acetic acid solution. Fractional crystallization of the resultant mixture gave a pure di-2-hydroxyethylamino derivative, m.p. 185–187°, in about 10 per cent yield based on the mixed nitro compounds. It has not proved possible to resolve the mixed nitro or amino compounds but the isolation of the least soluble hydroxyethylated product was relatively easy. The product, m.p. 185–187°, was converted into the di-2-chloroethylamino-hydantoin and amino acid derivatives in the usual manner.

As resolution was not possible before the hydroxyethylamino stage the orientation of the tetralin derivative could not be established by oxidation to a nitrophthalic acid. Alkaline hydrolysis of the product, m.p. 185–187°, afforded 2-amino-*x*-di-2′-hydroxyethylamino-1:2:3:4-tetrahydro-2-naphthoic acid but an attempt to oxidize this to the tetralone for eventual conversion into a naphthalene derivative was unsuccessful. It should be possible to decide whether the di-2-chloroethylamino- group in the hydantoin-5-spiro-2′-derivative is in the α or β position relative to the saturated ring by determining the rate of hydrolysis of the chlorine atoms under standard conditions.[4] For example, the extent of hydrolysis of the α-derivative (VIII) is 86 per cent and of the β-derivative (IX) is 44 per cent.[5] The hydantoin ring has a somewhat deactivating effect on chemical reactivity as shown by the rates of hydrolysis of CB 1718, CB 1739, and CB 1744 (14, 18·5, and 23·5 per cent, respectively, Table 1) all of which are definitely β-derivatives. Since the rate of hydrolysis of the hydantoin-5-spiro-2′- derivative is 67 per cent it is almost certainly an α-derivative. This conclusion is supported by the infra-red spectrum of CB 1740 which



(VIII)



(IX)

shows no maximum in the range 800–860 cm^{-1} characteristic of a 1:2:4-trisubstituted benzene derivative[6]: a peak at 820 cm^{-1} is exhibited by the β-derivative CB 1743.

At the moment it is not possible to distinguish between the 5-and 8-position for the di-2-chloroethylamino group.

Hydantoin-5-spiro-1′-(6′-aminoindane)

6-Amino-1-indanone[7] (19·0 g), ethanol (210 ml), water (210 ml), sodium cyanide (12·5 g) and ammonium carbonate (115 g) were stirred together at 60° for 8 hr. The solution was then concentrated to half bulk. The crystals which separated from the cooled solution were collected and a second crop obtained by adjusting the pH of the filtrate to 8. The hydantoin formed pale yellow prisms (26·3 g), m.p. 260° (decomp.), when crystallized from ethanol (Found: C, 61·2; H, 5·1; N, 19·3. Calc. for $C_{11}H_{11}N_3O_2$: C, 60·8; H, 5·1; N, 19·4%).

Hydantoin-5-spiro-1′-(6′-di-2″-hydroxyethylaminoindane)

Hydantoin-5-spiro-1′-(6′-aminoindane) (26·0 g), acetic acid (150 ml), water (150 ml), and ethylene oxide (50 ml) were stirred together at room temperature for 16 hr. The mixture was concentrated to 75 ml under reduced pressure and water (250 ml) was added. The crystalline precipitate was filtered off, washed with water, and dried. The *hydantoin* (29·1 g) formed plates, m.p. 197–199°, from water (Found: C, 59·1; H, 6·2; N, 13·7. Calc. for $C_{15}H_{19}N_3O_4$: C, 59·0; H, 6·3; N, 13·8%).

Hydantoin-5-spiro-1′-(6′-di-2″-chloroethylaminoindane)

Hydantoin-5-spiro-1′-(6′-di-2″-hydroxyethylaminoindane) (5·06 g) was stirred with phosphoryl chloride (40 ml) at 70 °C for 1½ hr. The excess reagent was then removed under reduced pressure and the residue was heated with concentrated hydrochloric acid (10 ml) on a steam bath for 5 min. The resulting solution was stirred into cold saturated aqueous sodium acetate (200 ml) which was then extracted with ether followed by chloroform. The solid (2·42 g) obtained on evaporating the dried extracts was purified by passing an acetone solution through a column of activated alumina and washing the column with fresh acetone. The eluates contained the *hydantoin* which formed needles, m.p. 199°, from acetone-light petroleum (b.p. 60–80°) (Found: C, 52·9; H, 5·1; Cl, 20·6; N, 12·4. Calc. for $C_{15}H_{17}Cl_2N_3O_2$: C, 52·6; H, 5·0; Cl, 20·7; N, 12·3%).

1-*Amino-6-di-2′-chloroethylaminoindane*-1-*carboxylic acid*

A solution of hydantoin-5-spiro-1′-(6′-di-2″-chloroethylaminoindane) (1·98 g) in concentrated hydrochloric acid (20 ml) was heated in a sealed tube at 160° for 2 hr. The solution was then boiled with charcoal, filtered, concentrated and stirred with cold saturated aqueous sodium acetate (35 ml) and the precipitated solid was collected by filtration. After washing with a little water and then acetone the amino acid, which was in the form of small prisms, darkened on heating to 190° and frothed at 220°. (Found: C, 50·0; H, 6·0; Cl, 20·7; N, 8·2. Calc. for $C_{14}H_{17}Cl_2N_2O_2.H_2O$: C, 50·2; H, 6·0; Cl, 21·2; N, 8·4%).

Hydantoin-5-spiro-2′-indane

Indan-2-one[8] (23·1 g), ethanol (420 ml), water (420 ml), ammonium carbonate (210 g), and sodium cyanide (25·2 g) were heated together at 55–60° for 10 hr. The solid which separated on cooling was collected by filtration and a second crop was obtained by concentrating the filtrate to half bulk and neutralizing with hydrochloric acid. The *hydantoin* formed needles, m.p. 260–267°, from ethanol; yield 19·0 g. (Found: C, 65·5; H, 4·9; N, 13·6. Calc. for $C_{11}H_{10}N_2O_2$: C, 65·3; H, 5·0; N, 13·9%).

3-*Ethyl-hydantoin-5-spiro-2′-(5′-nitroindane)*

A solution of hydantoin-5-spiro-2′-indane (18·6 g) in concentrated nitric acid (200 ml, d. 1·40) was kept at 30 °C for 1 hr and then poured into ice-water (1 l.). The pale yellow crystalline solid (20·3 g) which separated was collected, washed with water, and dried. The nitration mixture (22·2 g), m.p. 235–240 °C (decomp.), was dissolved in sodium hydroxide (45 ml, 2 N), water (25 ml), and ethanol (50 ml). After removing most of the solvents under reduced pressure the residue was dried by azetropic distillation with benzene. The dark orange powder was dissolved in hot ethanol (800 ml) and potassium iodide (5 g) and ethyl bromide (50 ml) were added and the mixture was heated under reflux for 5 hr. The residue obtained on evaporation was dissolved in ethyl acetate (500 ml) and washed successively with water, sodium hydroxide (2 N), and water and then dried over anhydrous sodium sulphate. Removal of the solvent gave a pale brown powder which was chromatographed on a column of activated alumina, elution being effected with ethyl acetate. The eluates contained a white solid which after being crystallized several times from benzene and methanol afforded the 3-*ethyl-hydantoin* as needles, m.p. 160 °C (yield 9·1 g) (Found: C, 56·8; H, 4·9; N, 14·9. Calc. for $C_{13}H_{13}N_3O_4$ C, 56·7; H, 4·8; N, 15·3%).

The ethylhydantoin (75 mg) was dissolved in concentrated sulphuric acid (1 ml) and potassium dichromate (200 mg) was added with stirring. After keeping at 45 °C for 2 hr iced water was added and the solution was extracted with *n*-butanol. The extract was shaken with aqueous sodium hydrogen carbonate and this alkaline solution was extracted with fresh butanol. The acid obtained on acidification of the aqueous layer was subjected to paper chromatography in *n*-propanol:0·88 ammonia:water (7 : 1 : 2) with 3-nitro- and 4-nitro-phthalic acids as reference compounds. The acids were detected by spraying with Universal Indicator solution (pink spots). R_f values were 3-nitrophthalic acid, 0·43; 4-nitrophthalic acid, 0·50, oxidation product from the ethylhydantoin, 0·50. The infra-red spectrum of the oxidation product was identical with that of 4-nitrophthalic acid but different from that of 3-nitrophthalic acid.

3-*Ethyl-hydantoin*-5-*spiro*-2′-(5′-*aminoindane*)

3-Ethyl-hydantoin-5-spiro-2′-(5′-nitroindane) (15·8 g) in hot ethanol (250 ml) was hydrogenated over a palladium–charcoal catalyst (5% Pd). The hot solution was filtered and refrigerated giving the *amino compound* (11·7 g) as plates, m.p. 128–129 °C (Found: C, 63·5; H, 6·2; N, 16·9. Calc. for $C_{13}H_{15}N_3O_2$: C, 63·7; H, 6·2; N, 17·1%).

3-*Ethyl-hydantoin*-5-*spiro*-2′-(5′-*di*-2″-*hydroxyethylaminoindane*)

The above amino compound (3·02 g) suspended in water (50 ml) containing acetic acid (3 ml) and ethylene oxide (10 ml) was stirred at room temperature overnight. After concentrating to low bulk under reduced pressure saturated aqueous sodium hydrogen carbonate was added and the solid product (3·59 g) was collected, washed with water, and dried. The *monohydrate* of the *di-2-hydroxyethyl derivative* formed plates, m.p. 88 °C, from water (Found: C, 58·1; H, 6·9; N, 11·9. Calc. for $C_{17}H_{23}N_3O_4.H_2O$: C, 58·1; H, 7·2; N, 12·0%).

3-*Ethyl-hydantoin*-5-*spiro*-2′-(5′-*di*-2″-*chloroethylaminoindane*)

The di-2-hydroxyethylamino derivative (7·2 g) in phosphoryl chloride (75 ml) was stirred at 65–70° for 1½ hr. After removing the excess phosphoryl chloride under reduced pressure the residue was heated on a steam bath with concentrated hydrochloric acid (30 ml) until completely dissolved. The precipitate which formed when this solution was poured into saturated aqueous sodium acetate was extracted into ethyl acetate and the washed extract was dried over sodium sulphate. The product was chromatographed on activated alumina, elution being effected with ethyl acetate–acetone (1 : 1). Evaporation of the appropriate eluates gave the *di-2-chloroethylamino*-derivative (4·91 g), which formed small prisms, m.p. 158 °C, from acetone-light petroleum (b.p. 40–60°) (Found: C, 55·1; H, 5·7; Cl, 19·2; N, 11·1. Calc. for $C_{17}H_{21}Cl_2N_3O_2$: C, 55·1; H, 5·7; Cl, 19·2; N, 11·4%).

2-*Amino*-5-*di*-2′-*chloroethylaminoindane*-2-*carboxylic acid*

A solution of 3-ethyl-hydantoin-5-spiro-2′-(5′-di-2″-chloroethylaminoindane) (1·5 g) in concentrated hydrochloric acid (15 ml) was heated at 160 °C for 2 hr. The solution was then boiled with charcoal, filtered, concentrated to low bulk, and then added with stirring to saturated aqueous sodium acetate (25 ml). The precipitate (0·79 g) was washed with water and acetone and then dried. The *amino acid* formed small prisms, m.p. 225° (decomp.), from methanol–ether (Found: C, 53·1; H, 5·8; Cl, 21·4; N, 8·7. Calc. for $C_{14}H_{18}Cl_2N_2O_2$: C, 53·0; H, 5·7; Cl, 22·4; N, 8·8%).

Hydantoin-5-*spiro*-2′-(x-*di*-2″-*hydroxyethylamino*-1′:2′:3′:4′-*tetrahydronaphthalene*

Hydantoin-5-spiro-2′-(1′:2′:3′:4-tetrahydronaphthalene) — hydantoin from β-tetralone—(20 g, m.p. 265 °C) was added during ¾ hr to stirred concentrated nitric acid (200 ml, d. 1·42). The temperature, which had remained at 20–23 °C during the addition was raised to 30 °C for 1 hr. Water (300 ml) was then slowly added and a mixture of mono-nitro compounds separated as a granular solid (22 g), m.p. 230–240 °C (Found: C, 55·1; H, 4·3. Calc. for $C_{12}H_{11}O_4N_3$: C, 55·1; H, 4·2%). This product was dissolved in dimethylformamide (120 ml) and shaken in an atmosphere of hydrogen over a palladium–charcoal catalyst (5% Pd). Reduction of the nitro group was complete in 10 hr when the filtered solution was evaporated to dryness and extracted with dilute hydrochloric acid (2 N). On adding ammonia to this extract a mixture of mono-amino compounds (14·8 g) separated. Small plates, m.p. above 300°, were obtained when this material was dissolved in cold aqueous ammonium hydroxide and the solution was heated to boiling. (Found: C, 62·3; H, 5·5; N, 18·4. Calc. for $C_{12}H_{13}O_2N_3$: C, 62·3; H, 5·7; N, 18·2%). The mixed amines (7 g), ethylene oxide (20 ml), glacial acetic acid (30 ml), and water (30 ml) were stirred at room temperature for 16 hr. The solid, obtained after evaporating the solution to low bulk and triturating with acetone, was crystallized from water several times when long prismatic needles (1·8 g), m.p. 185–187 °C, of the *dihydroxyethyl* compound were obtained (Found: air dry specimen, C, 57·7; H, 6·4; N, 12·4; specimen dried at 130 °C/0·02 mm for 6 hr, C, 58·8; H, 6·7; N, 12·5. Calc. for $C_{16}H_{21}O_4N_3.H_2O$: C, 57·0; H, 6·8; N, 12·5. Calc. for $C_{16}H_{21}O_4N_3.\frac{1}{2}H_2O$: C, 58·5; H, 6·8; N, 12·8%).

2-*Amino*-x-*di*-2′-*hydroxyethylamino*-1:2:3:4-*tetrahydro*-2-*naphthoic acid*

The hydroxyethylamino-hydantoin (4·2 g) and barium hydroxide octahydrate (12·0 g) in water (80 ml) were heated at 160 °C for 2½ hr in a pressure bottle. The cooled solution was filtered and then filtrate treated with an excess of ammonium carbonate and refiltered. Evaporation of this filtrate gave a solid (2 g) which was crystallized by dissolving in cold water, adding acetone until a faint turbidity formed and then leaving to stand. Flattened needles of the *amino acid*, m.p. 218–129 °C, slowly separated (Found: C, 61·3; H, 7·7; N, 9·4. Calc. for $C_{15}H_{22}O_4N_2$: C, 61·2; H, 7·5; N, 9·5%).

Hydantoin-5-*spiro*-2′-(x-*di*-2″-*chloroethylamino*-1′:2′:3′:4′-*tetrahydronaphthalene*)

After stirring the above hydantoin (1 g) in phosphoryl chloride (10 ml) at 50 °C for 5 hr a homogeneous solution was obtained. This was evaporated to dryness under reduced pressure and again after the addition of dry benzene. The residue was dissolved in concentrated hydrochloric acid (10 ml) and the solution again evaporated. An acetone solution of the product was passed through a column of activated alumina containing a small upper layer of charcoal. Addition of light petroleum (b.p. 40–60°) to the colourless acetone eluates caused the precipitation of the *chloroethyl* derivative as an amorphous powder (m.p. 186–187·5°) (Found: C, 54·3; H, 5·4; N, 11·5. Calc. for $C_{16}H_{19}O_2N_3Cl_2$: C, 53·9; H, 5·4; N, 11·8%).

2-*Amino*-x-*di*-2′-*chloroethylamino*-1:2:3:4-*tetrahydronaphthoic acid*

A solution of the chloroethyl derivative (1 g) in concentrated hydrochloric acid (10 ml) was heated in a sealed tube at 160–170 °C for 2 hr. After evaporation to low bulk on a steam bath the addition of solid ammonium acetate caused the separation of a granular solid (500 mg). Purification was difficult but analytically acceptable material was obtained by dissolution in dilute hydrochloric acid and gradual reprecipitation by adding saturated aqueous sodium acetate. The amorphous *amino acid* had no definite m.p.; it shrinks between 185 and 190° and froths at about 260° (Found: C, 54·2; H, 6·3. Calc. for $C_{15}H_{20}O_2N_2Cl_2$: C, 54·4; H, 6·1%).

Hydrolysis rates

The assessment of the relative chemical reactivity of the di-2-chloroethylaminoaryl derivatives was made by a refinement of the method described by Ross[4]. The compound (0·0005 mole) was dissolved in 50 ml or 100 ml aqueous acetone (50% v/v) according to its solubility and the pH of the solution was adjusted to 7 by adding 0·1 N sodium hydroxide using a glass electrode system. The solution was heated at boiling point for ½ hr then cooled and the pH again adjusted to 7. From the amount of 0·1 N alkali required to restore the pH of the solution to 7 the extent of hydrolysis of the chlorine atoms could be calculated. The results are given in Tables 1 and 2.

Toxicity determinations

Preliminary toxicity tests were carried out on groups of three female Wistar rats. Unless otherwise stated all injections of the arachis oil solution or suspension of the drug were made by the intraperitoneal route and the animals were observed for 14 days. In four cases a closer assessment of the LD$_{50}$ was made by using four groups of four male rats with logarithmically graded doses (cf. Weil[9]).

Tumour inhibition studies

The tests for inhibition of the Walker rat carcinoma 256 were carried out and assessed exactly as described by Connors *et al.*[10]

RESULTS

The results of the various determinations made with the di-2-chloroethylaminoarylhydantoins and derived amino acids are given in Tables 1 and 2.

TABLE 1. PROPERTIES OF SOME DI-2-CHLOROETHYLAMINOARYLHYDANTOINS

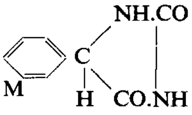
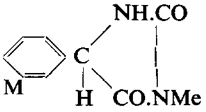
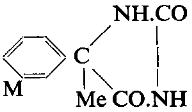
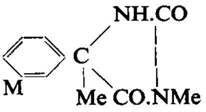
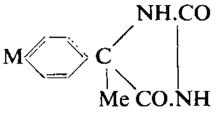
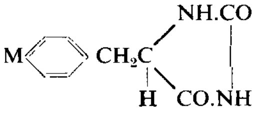
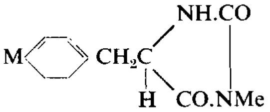
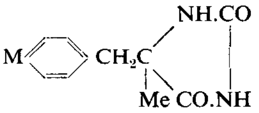
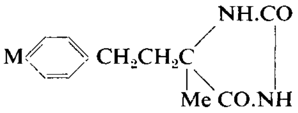
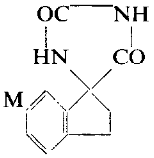
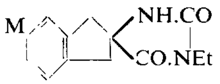
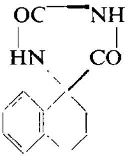
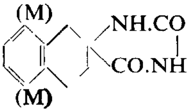
CB No.	Structure**	Hydrolysis Rate* (%)	Toxicity data Dose mg/kg†	Toxicity data Deaths‡	Activity against Walker tumour Dose mg/kg†	Activity against Walker tumour C/T ratio§
CB 1655		10·3	50 25	3/3 1/3	15 5	∞ 10
CB 1657		10·9	50 37·5 25	3/3 2/3 0/3	15	11
CB 1678		13·4	100 50	3/3 1/3	25	13·2
CB 1687		7·5	50	0/3	50	2·7

TABLE 1.—*continued*

CB No.	Structure**	Hydrolysis Rate* (%)	Toxicity data Dose mg/kg†	Toxicity data Deaths‡	Activity against Walker tumour Dose mg/kg†	Activity against Walker tumour C/T ratio§
CB 1652		6·5	250	0/3	250 100	∞ 24
CB 1648		20·5	125 50 25	3/3 3/3 0/3	25	150
CB 1658		14·8	150 100	0/3 0/3	150 75 25	∞ ∞ 2·7
CB 1726		20	50 25	0/3 0/3	25 2	∞ 1
CB 1675		24	100 50	3/3 1/3	25 10	95 2·1
CB 1739		18·5	20 16·7 13·9 11·5 LD$_{50}$ 13·8 (males)	4/4 4/4 2/4 0/4	10 5 2	∞ ∞ 22
CB 1744		24	50 40 20 10	3/3 3/3 0/3 0/3	20 10	64 3·7
CB 1718		14	124 103 86 72 LD$_{50}$ 95 (males)	4/4 4/4 0/4 0/4	24 10	∞ 12
CB 1741		67	25 10	3/3 1/3	5	2·1

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* Release of hydrogen ions on refluxing for ½ hr in 1:1 acetone–water. c = 0·01 M or 0·005 M according to solubility.

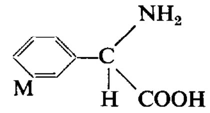
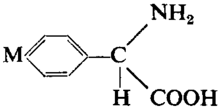
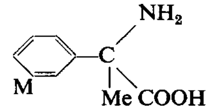
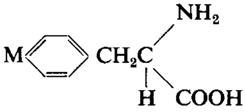
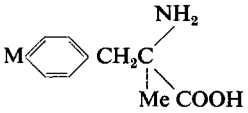
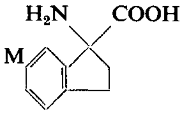
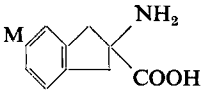
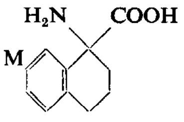
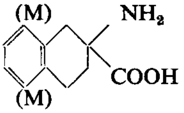
† Intraperitoneal injection in arachis oil.

‡ Female Wistar rats (unless otherwise stated): deaths within 14 days

§ Total weight of tumours in control group/total weight of tumours in treated group (six animals per group).

** M = —N(CH$_2$CH$_2$Cl)$_2$.

TABLE 2. PROPERTIES OF SOME DI-2-CHLOROETHYLAMINOARYL-DL-AMINO ACIDS

			Toxicity data		Activity against Walker tumour	
CB No.	Structure	Hydrolysis Rate (%)	Dose mg/kg	Deaths	Dose mg/kg	C/T ratio
CB 1656		12	25	0/3	15 5	∞ 47
CB 1447		10	25	0/3	10	∞
CB 1724		12	25	0/3	25 10 5	∞ ∞ 2
CB 3007		22	16	2/4	2 0·5	∞ 4·3
CB 1746		20	70 60 40	3/3 1/3 0/3	20 5 2	∞ 11 1
CB 1743		23 (H^+) 26 (Cl^-)	78·1 62·5 50 40 LD_{50} 50 (males)	4/4 4/4 2/4 0/4	20 10 5 2	∞ ∞ 3 1
CB 1748		22	12 10 5	1/3 0/3 0/3	2 5	6·5 ∞
CB 1723		22·5	49 29 17 10 LD_{50} 17 (males)	4/4 4/4 2/4 0/4	10 5 4·25 2	∞ ∞ 10 1·5
CB 1740		69	10 5	3/3 0/3	2	4

Chemical reactivity

Generally the change in the chemical reactivity of the chlorine atoms in passing from the hydantoin to the corresponding amino acid is not significant but in one case this is appreciable, namely, CB 1718 → CB 1723 (14→22·5 per cent). A smaller change is observed in the conversions CB 1739 → CB 1743 (18·5 → 23 per cent) and CB 1658 → CB 3007 (14·8 → 22 per cent). 3-N-Methylation on the hydantoin ring has little effect on the reactivity in the conversion of CB 1655 → CB 1657 (10·3 → 10·9 per cent) but reduces reactivity in the case of CB 1678 → CB 1687 (13·4 → 7·5 per cent) and of CB 1648 → CB 1658 (20·5 → 14·8 per cent). As a result the *in vivo* transformation of these last two N-methylated hydantoins should be accompanied by a larger increase in biological activity than would be encountered for the unmethylated derivatives. α-C-Methylation has little effect on chemical reactivity (compare CB 1447 → CB 1724, 10 → 12 per cent; CB 3007 → CB 1746, 22 → 20 per cent; CB 1655 → CB 1678, 10·3 → 13·4 per cent; CB 1648 → CB 1726, 20·5 → 20 per cent).

Toxicity

There is insufficient data to attempt a detailed correlation between toxicity and chemical reactivity but there is an indication that, as would be expected, the most reactive compounds are also the most toxic. The amino acid is generally more toxic than the related hydantoin, the only exception being the pair CB 1743 and CB 1739 (LD_{50} 50 and 13·8 mg/kg respectively). 3-N-Methylation appears to reduce the toxicity of hydantoins—this is quite clear in the case of CB 1648 → CB 1658 and is also suggested in the pairs CB 1655, CB 1657 and CB 1678, CB 1687. 5-C-Methylation of hydantoins and the comparable α-C-methylation of the derived amino acid definitely reduces toxicity (compare CB 1648 → CB 1726 and CB 3007 → CB 1746). The amino acid CB 1743 is remarkable in causing significant atrophy of the spleen when administered as a single dose of 2 mg/kg intraperitoneally in arachis oil solution. After such treatment the combined weights of spleen in six rats of average weight 240 g was 7·2 g compared with a value of 15·9 g for six untreated animals.

Anti-tumour activity

Most of the di-2-chloroethylaminoaryl derivatives included in this paper will cause complete inhibition of the growth of the 1-day-old Walker tumour when administered in adequate amount. The low activity of the two compounds CB 1740 and CB 1741 might seem surprising in view of their high reactivity and considerable toxicity. However, it has been the experience in this Institute that the more reactive nitrogen mustards are not very active anti-tumour agents when given as a single dose on the day following tumour implantation. Numerous aliphatic derivatives, including HN_2, are almost ineffective in this simple test but are active when the drug is given in repeated daily doses. A recent example of this fact was encountered with the surface active compound:

$$(ClCH_2CH_2)_2N(CH_2)_{10}COOMe$$

which is inactive when given as a single dose of 10 mg/kg but which gave a C/T ratio of 4 when given as a daily dose of 5 mg/kg.

One of the objects of this work was to examine α-methylated derivatives of the very effective di-2-chloroethylaminophenylalkaneamino acid series in view of the statement that such alkyl substituted amino acids tend to concentrate within cells.[11] However, it is clear that the introduction of an α-methyl group into CB 3007 (merphalan, sarcolysin) giving CB 1746 has considerably reduced the anti-tumour activity.

DISCUSSION

Potentiation of the activity of di-2-chloroethylaminoaryl hydantoins *in vivo* could occur by two mechanisms: (a) by the generation of an amino acid which would be more active because of favourable transport characteristics, and (b) by the formation of a derivative which is chemically more reactive. The present study has shown that such potentiation is possible with certain structures, a particularly favourable situation arises with CB 1718 which can generate the chemically more reactive and presumably better transported amino acid CB 1723. Of course, selectivity of action against a particular neoplasm would only be achieved if such tissue had a greater ability to effect the transformation of hydantoin to amino acid. In the absence of a more detailed study of the biochemical differences between normal and cancer cells the approach to chemotherapy must necessarily be rather empirical at this time.

In this admittedly preliminary study it is not possible to assess accurately the relative chemotherapeutic index of a hydantoin and the corresponding amino acid with regard to the Walker tumour—this index being defined as the ratio of LD_{50} dose to the dose required to cause complete inhibition of tumour growth. Taking an overall view of the results now presented the index for the hydantoins falls in the range 2–4 and the index for the derived amino acids in the range 4–10.

The relatively low value of the index for the hydantoins with respect to the Walker tumour argues against selective hydrolysis within this tumour. In view of established differences in the enzymic make-up of various tumours it is clearly desirable to extend the testing of these hydantoins against a wider range of neoplasms.

For this reason some preliminary testing of the hydantoin, CB 1718, against two solid tumours and two leukaemias has been undertaken. When given as five daily intraperitoneal injections of 150 mg/kg to mice bearing sarcoma 180 a C/T ratio of 27 was obtained and when the compound was similarly administered at a daily dose of 110 mg/kg to mice bearing the adenocarcinoma 755 the C/T ratio was 3. Rats grafted with the Furth leukaemia, if untreated, survive on an average for 15 days; this is increased to 25 days if the animals receive a single intraperitoneal injection of 25 mg/kg of CB 1718 (all doses made up in arachis oil) on the third day after grafting. The hydantoin showed no activity against the mouse lymphoid leukaemia L 1210.

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