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THE PHARMACOLOGY AND TUMOUR GROWTH INHIBITORY ACTIVITY OF 1-AMINO*CYCLO*PENTANE-1-CARBOXYLIC ACID AND RELATED COMPOUNDS

T. A. CONNORS, L. A. ELSON, A. HADDOW and W. C. J. ROSS

The Chester Beatty Research Institute, Institute of Cancer Research, The Royal Cancer Hospital, London, S.W.3

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Abstract—The tumour growth inhibitory activity and toxicity of a number of alicyclic α-amino acids and related compounds has been investigated. Compounds studied include amino acid derivatives of *cyclo*propane, *cyclo*butane, *cyclo*pentane, *cyclo*hexane, *cyclo*heptane, and *cyclo*octane. Significant anti-tumour activity was only exhibited by compounds closely related to 1-amino*cyclo*pentane-1-carboxylic acid. The pharmacological properties of a number of the compounds examined are discussed.

INTRODUCTION

THE tumour growth inhibitory activity of 1-amino*cyclo*pentane-1-carboxylic acid has been briefly reported.[1] Some pharmacological, toxicological, and anti-tumour properties of a series of derivatives and analogues of this active cyclic amino acid are dealt with in the present communication. The preparation and chemical properties of the new compounds examined have been reported elsewhere.[2]

EXPERIMENTAL

Materials

All compounds tested were synthesised in our laboratories. We are indebted to Dr. Howard Bond of the Cancer Chemotherapy National Service Center of the National Institutes of Health, Bethesda, U.S.A., for making available an extra supply of 1-amino*cyclo*pentane-1-carboxylic acid. For the biological tests the compounds, unless otherwise stated, were administered to the rats by the intraperitoneal route and were dissolved in water or suspended in arachis oil.

Animals and diets

Male and female rats from an inbred Wistar colony were used. Their normal food consisted of "rat cake", a commercial product of the following composition:

	(%)
Fine bran	17·4
Ground wheat	17·4
Sussex ground oats	17·4
Ground maize	8·7
Ground barley	8·7
White fish meal	4·8
Meat and bone meal	9·6
Dried skimmed milk	14·0
Dried yeast	1·2
Salt	0·4
Cod liver oil	0·4

In some toxicity experiments animals were fed on a "soft mash" diet consisting of:

	(%)
Water-soaked bread	75·0
Milk powder	6·0
Casein	5·0
Bemax	4·0
Bran	4·0
Dried yeast	3·0
Salt	1·0
Cod liver oil	1·0
Chalk	1·0

5, 10 and 20 per cent protein diets were also used in some tests.*

The animals undergoing the Walker tumour tests were maintained on a varied diet which has been used routinely in our animal colony for many years. Over a 7-day period they have "rat cake" on two non-consecutive days and on other days have bread and milk, bread and margarine, bread and marmite, bread and cod liver oil, and oats.

Toxicity determinations

Preliminary toxicity tests were carried out on groups of three female rats. The animals were about 6–7 weeks old and weighed about 200 g. They were fed on rat cake diets only and the compounds were administered intraperitoneally in water except where otherwise stated (see Tables 1–6). Each animal was weighed daily for at least 4 days before injection of a test compound. Weighing was continued for a further 12–14 days and the growth curve plotted. Weight loss (L_1) was assessed as described by Elson[3] by comparing the actual weight reached with that which would have been attained if the animal had continued its pre-injection steady rate of growth.

* The composition of the 5 per cent protein diet is: casein 5 per cent, starch 85 per cent, Bemax 2·5 per cent, margarine 5 per cent, chalk 0·5 per cent, Salt mixture 1 per cent, Cod liver oil 1 per cent. For the 10 and 20 per cent protein diets the casein is increased by 5 and 15 per cent and starch reduced by the corresponding amounts.

Post-mortem examinations were carried out on animals which died and on survivors killed after the 12–15 day test period. Histological examinations were made on sections of various organs in some cases.

Tumour inhibition experiments

The tests for inhibition of Walker rat carcinoma 256 were carried out on male rats essentially as described by Haddow *et al.*,[4] 6 animals being used in each of the control and test groups. Treatment was started on the day following implantation of the tumour and except where otherwise stated (see Tables 1–6) the compounds were injected intraperitoneally in aqueous solution or occasionally suspended in arachis oil. A few experiments have also been carried out with oral administration of aqueous solutions. In assessment of the degree of inhibition the ratio C/T refers to the total weight of the tumours of the control group divided by that of the treated group when the animals are killed and the tumours dissected out and weighed: this is usually done 10 - 12 days after implantation.

Blood counts

Serial blood counts have been carried out on rats treated with 1-amino*cyclo*pentane-1-carboxylic acid (CB 1639). Blood was taken from a tail vein direct into the pipette in which it was diluted in the usual manner. Haemoglobin was estimated photocolorimetrically on a suitably diluted solution.

RESULTS

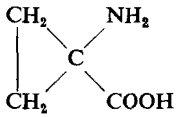
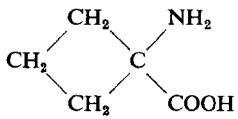
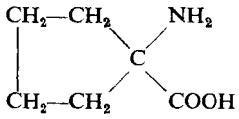
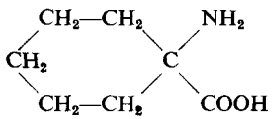
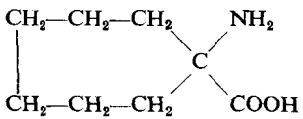
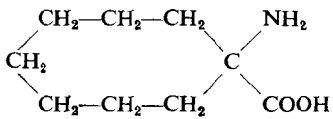
Tumour growth inhibition and toxicity

Table 1 shows toxicity data and activity against the Walker rat carcinoma 256 of a series of alicyclic α-amino acids, in which the ring size has been varied from 3 to 8 carbon atoms. Tumour growth inhibitory activity has only been shown by the *cyclo*pentane derivative (CB 1639) which is also the most toxic compound of the series.

Although a single dose of 250 mg of CB 1639 per kg caused prolonged growth inhibition in normal rats this dose had no appreciable effect on tumour growth. Six consecutive daily doses of 125 mg/kg started on the day following implantation and given either intraperitoneally or by forced feeding caused definite inhibition of the growth of the tumour: this effect was still apparent, though less marked, with a dose of 75 mg/kg given similarly during 8 days. In one experiment the transplanted Walker carcinoma was allowed to grow for 7 days before treatment began; no inhibition of growth occurred when a dose of 75 mg/kg was given on the 6 following days. CB 1639 also checked the growth of the more resistant Crocker sarcoma 180 in the mouse. A C/T ratio of 5·7 was obtained in groups of 5 animals—the treated animals receiving five daily injections of 2 mg per 25 g mouse of amino acid in 0·2 ml of water. The compound was toxic to mice at double this dose. When C^- Bagg Albino mice bearing a first transplant from a C^+ mouse mammary tumour were treated with eleven doses of 2 mg of CB 1639 per mouse given on alternate days a C/T ratio of 2·85 was obtained.

Table 2 shows the results obtained with a few ring substituted derivatives of the *cyclo*pentane and *cyclo*hexane amino acids. The introduction of a carboxyl group into CB 1639 leads to the lowering of toxicity but also to the loss of anti-tumour

TABLE 1

Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	C/T	Single dose (mg/kg)	Deaths within 2 weeks
CB. 1703	1-Aminocyclopropanecarboxylic acid		250	5 (alternate)	−ve		500	0/3
1700	1-Aminocyclobutanecarboxylic acid		500	5 (alternate)	−ve		500	0/3
1639	1-Aminocyclopentanecarboxylic acid		500	3	toxic		♀1000	3/3
			250	1	low	1·29	♀ 750	3/3
			125	6	+ve	30·5	♀ 500	3/3
			125 f.f.*	6	+ve	10·3	♀ 375	7/8
			75	8	+ve	3·0	♀ 250	1/6
			75 f.f.	8	+ve	3·6	♀ 200	0/3
							♂ 250	7/9
							♂ 150	0/6
1641	1-Aminocyclohexanecarboxylic acid		500	12	−ve		1000	0/3
							500	0/3
1692	1-Aminocycloheptanecarboxylic acid		500	4 (alternate)	−ve		500	0/3
1714	1-Aminocyclooctanecarboxylic acid		500	4 (alternate)	−ve		500	0/3

* Forced feeding

TABLE 2

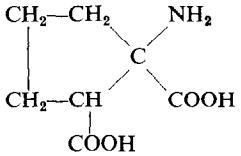
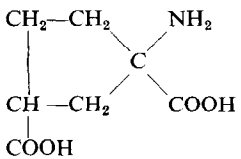
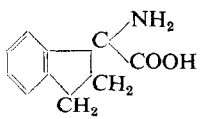
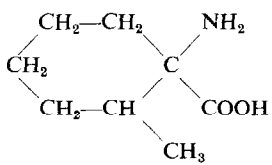
Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	*C/T*	Single dose (mg/kg)	Deaths within 2 weeks
1701	1-Amino*cyclo*pentane-1 : 2-dicarboxylic acid		500	5 (alternate)	−ve		500	0/3
1712	1-Amino*cyclo*pentane-1 : 3-dicarboxylic acid		500	4 (alternate)	−ve		500	0/3
1696	1-Amino-2 : 3-benz*cyclo*pentane-1-carboxylic acid		250	8	low	1·3		
1645	1-Amino-2-methyl*cyclo*hexane-carboxylic acid		500	10	−ve		500	0/3

TABLE 2—*continued.*

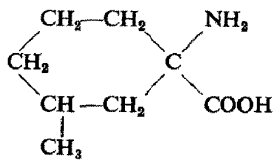
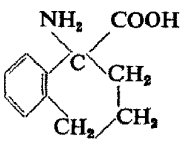
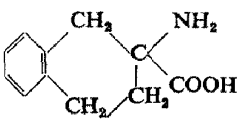
Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	*C/T*	Single dose (mg/kg)	Deaths within 2 weeks
1638	1-Amino-3-methyl*cyclo*hexane-carboxylic acid		500	8	−ve		1000	0/3
1643	1-Amino-2 : 3-benz*cyclo*hexane-1-carboxylic acid		500	12	−ve		500	0/3
1647	1-Amino-3 : 4-benz*cyclo*hexane-1-carboxylic acid		500	10	−ve		500	0/3

TABLE 3

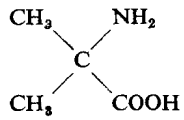
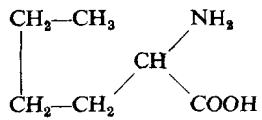
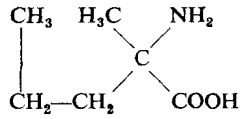
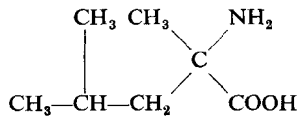
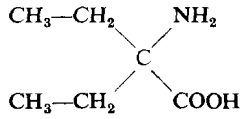
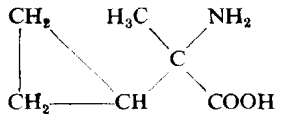
Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	*C/T*	Single dose (mg/kg)	Deaths within 2 weeks
CB 1637	α-Methylalanine		1000	9	−ve		2500	0/3
1688	1-Aminopentanecarboxylic acid (DL-norleucine)		250	9	−ve			
1685	2-Aminopentane-2-carboxylic acid		500	8	−ve		500	0/3
1640	α-Methyl-leucine		1000	10	−ve		1000	0/3
1686	3-Aminopentane-3-carboxylic acid		500	9	low	1·5	500	0/3
1653	α-*cyclo*Propylalanine		250	7	low	1·36	500 250 500 (oil) 250 (oil)	2/3 0/3 3/3 0/3

TABLE 4

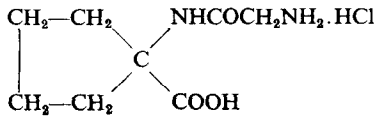
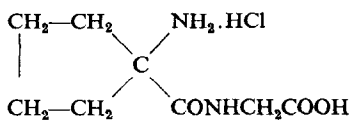
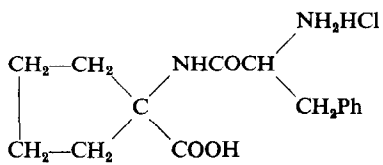
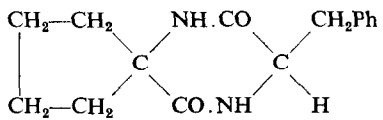
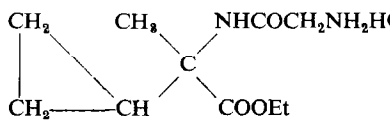
Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	*C/T*	Single dose (mg/kg)	Deaths within 2 weeks
CB 1659	Hydrochloride of glycyl-1-amino-*cyclo*pentane-1-carboxylic acid		250 125	6 8	+ve +ve	9 5·3	500	0/3 L_1 70 g
1668	Hydrochloride of 1-amino-1-(N-carboxymethyl)carboxyamido-*cyclo*pentane		125	10	+ve	7·8	500	0/3
1677	Hydrochloride of DL-phenylalanyl-1-amino*cyclo*pentanecarboxylic acid		100	8	−ve		250	0/3
1690	3-Benzyl-6 : 6-tetramethylene-2 : 5-dioxopiperazine		250	5 (alternate)	−ve		500	0/3
1706	Hydrochloride of glycyl-α-*cyclo*-propylalanine-ethyl ester		500	8	−ve		1000 500	0/3 0/3

TABLE 4—*continued.*

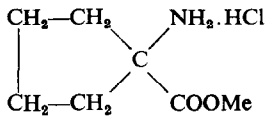
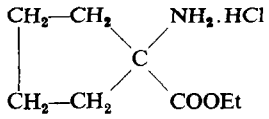
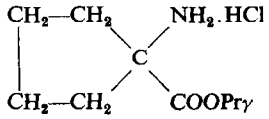
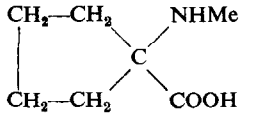
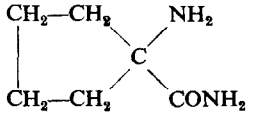
Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	*C/T*	Single (mg/kg) dose	Deaths within 2 weeks
1704	Hydrochloride of methyl-1-amino-*cyclo*pentanecarboxylate		250	5	Toxic		500	3/3
1691	Hydrochloride of ethyl-1-amino-*cyclo*pentanecarboxylate		375 (oil) 250	1 5	+ve Toxic	4·7	750 (oil) 500	0/3 L_1 44 g 1/3 L_1 100 g
1708	Hydrochloride of *iso*propyl-1-amino-*cyclo*pentanecarboxylate		250 125 50	5 6 10	Toxic Toxic +ve	2·3	500	0/3
1728	1-Methylamino*cyclo*pentane-carboxylic acid		250	1	−ve		375	0/3
1705	1-Amino-1-carbamido*cyclo*pentane		125	6	+ve	7·4	500 375 250	3/3 3/3 0/3

TABLE 4—*continued.*

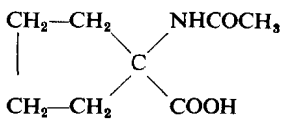
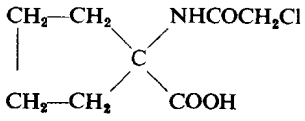
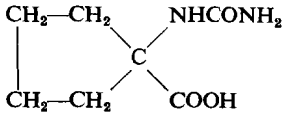
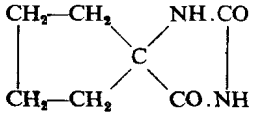
Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	*C/T*	Single dose (mg/kg)	Deaths within 2 weeks
1709	1-Acetylaminocyclopentane-carboxylic acid		250	9	low	1·3	500 250	0/3 0/3
1732	1-Chloracetylaminocyclopentane-carboxylic acid		500	1	−ve		500	0/3
1702	1-Ureidocyclopentanecarboxylic acid		500	9	−ve		500 (in bicarb.)	0/3
1683	5 : 5-Tetramethylenespirohydantoin		500 (oil)	8	−ve		1000 (oil)	0/3

TABLE 5

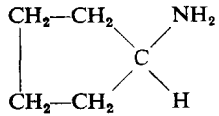
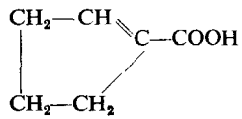
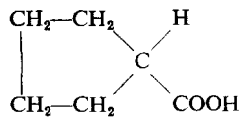
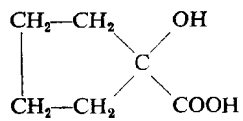
Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	C/T	Single dose (mg/kg)	Deaths within 2 weeks
1689	*cyclo*Pentylamine		25 (oil)	9	−ve		500 250 100 50	3/3 3/3 3/3 0/3 L_1 27 g
1684	*cyclo*Pent-1-enecarboxylic acid		500 (bicarb.)	8	−ve		500 (bicarb.) 250 (bicarb.) 125 (bicarb.) 250 (water)	0/3 0/3 0/3 2/3
1725	*cyclo*Pentanecarboxylic acid		500 (bicarb.)	8	−ve			
1693	1-Hydroxy*cyclo*pentanecarboxylic acid		250 125	2 7	toxic low	1·4	500	1/3

TABLE 5—*continued.*

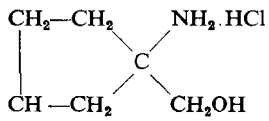
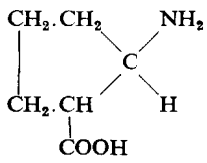
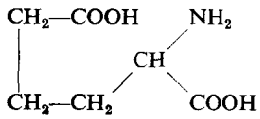
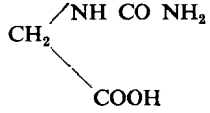
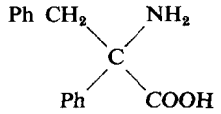
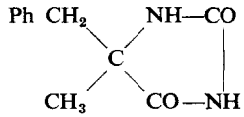
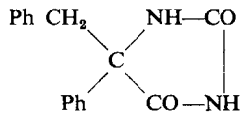
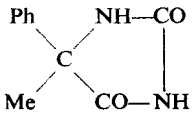
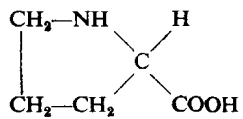
Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	*C/T*	Single dose (mg/kg)	Deaths within 2 weeks
1722	1-Hydroxymethylpentylamine hydrochloride		500	4	−ve			
1713	2-Amino*cyclo*pentanecarboxylic acid		250	9	−ve		500	1/5
1710	α-Aminoadipic acid		500 (bicarb.)	6	−ve		500	0/3

TABLE 6

Code no.	Compound	Structure	Activity against Walker tumour				Toxicity data	
			Dose (mg/kg)	Time (days)	Result	C/T	Single dose (mg/kg)	Deaths within 2 weeks
CB 1636	Hydantoic acid		125 f.f.	8	−ve		1000 500 250	3/3 2/3 0/3
1654	α : β-Diphenylalanine		500 (oil)	3	−ve		500 (oil)	0/3
1665	5-Benzyl-5-methylhydantoin		250 (oil)	7	−ve		500 (oil)	0/3
1715	5-Benzyl-5-phenylhydantoin		250	4 (alternate)	−ve		250	0/3
1664	5-Methyl-5-phenylhydantoin		125 (oil)	7	−ve		500 (oil) 250 (oil)	3/3 0/3
1707	L-Proline		500	6	−ve			

activity. The compound obtained by fusing a benzene ring on to the molecule of CB 1639—1-amino-2 : 3-benz*cyclo*pentane-1-carboxylic acid (CB 1696)—shows inhibitory activity of a very low order. No activity was shown by the methyl or benz-substituted cyclohexane amino acids.

Having established the unique character of the five-membered ring system it was then of interest to decide whether the cyclic structure was of importance—it will be realized that this work began as an investigation into α-substituted non-cyclic amino acids.[1] Accordingly compounds were examined (Table 3) which represented ring opening between C_1 and C_2 (CB 1688), C_2 and C_3 (CB 1685), and between C_3 and C_4 (CB 1686). Only the last compound showed any inhibitory activity and this was of a low order compared with the cyclic compound. A compound (CB 1653) in which ring closure of the five carbon chain was effected between C_2 and C_4 also showed slight anti-tumour activity. A single dose of 250 mg/kg of CB 1653 caused significant weight loss in the normal rat.

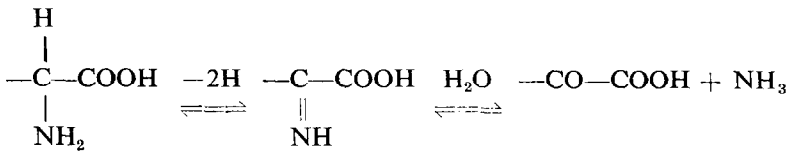
As it had now been established that appreciable activity was only associated with the *cyclo*pentane structure numerous derivatives of CB 1639 were next examined (Table 4). Since there is evidence[5] that the anti-metabolic activity of an amino acid analogue can be enhanced by incorporation into a peptide structure the preparation for test of two glycine and two phenylalanine peptides of 1-amino*cyclo*pentane-1-carboxylic acid was attempted.[2] Both glycine peptides (CB 1659 and CB 1668) showed activity comparable with that of the parent amino acid and were considerably less toxic. On the other hand, the phenylalanine peptide (CB 1677) and the corresponding cyclic dipeptide (CB 1690) showed no activity. A glycine peptide of *cyclo*propylalanine (CB 1706) was inactive against the transplanted tumour though this peptide retained the ability possessed by the parent acid (CB 1653) to retard the growth of the normal rat.

A number of derivatives of 1-amino*cyclo*pentane-1-carboxylic acid which could readily yield the amino acid *in vivo* was studied: it has been established that derivatives of cytotoxic agents with so-called latent activity[6] are often of interest since they constitute a transport form with physical properties differing from those of the parent agent.Three esters which would hydrolyse at different rates to give the amino acid were examined. The most readily hydrolysed methyl ester (CB 1704) proved to be the most toxic and the more stable *iso*propyl ester (CB 1708) the least toxic. In general there appeared to be no particular advantage in using the esters of CB 1639 as far as tumour growth inhibition was concerned.

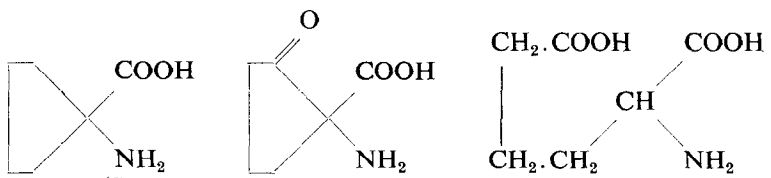
Two amides, in which the carboxyl group (CB 1705) or the amino group (CB 1709) were masked, were tested. The compound containing the free amino group was more toxic and exerted the greater effect on the tumour. Derivatives possessing a free amino group often show greater activity as indicated by the retention of activity in the glycine peptides and in similar peptides of melphalan.[7] It has been shown[8] that some hydantoins are hydrolysed *in vivo* to give the corresponding amino acids and accordingly the spirohydantoin (CB 1683) and the intermediate hydrolysis product (the ureido acid, CB 1702) were examined. These compounds proved to be ineffective and it can be assumed that no significant amounts of the amino acid were, in fact, formed.

The importance of the five-membered ring structure for anti-tumour activity prompted a consideration of differences between 1-amino*cyclo*pentane-1-carboxylic

acid and closely related but inactive homologues. One metabolic pathway followed by α-amino acids involves oxidation and reversible amination processes thus:



This pathway is not available for α-substituted α-amino acids since the formation of the imino acid is precluded. However β-oxidation, such as occurs with linear fatty acids, can proceed and by this route CB 1639 (I) would give the cyclic keto acid (II):



Ring fission of (II) should proceed readily to give α-aminoadipic acid (III), a compound which accumulates during abnormal lysine metabolism.[9] There is reason to believe that keto acids of type (II) derived from *cyclo*hexane would less readily undergo ring fission[10] whilst the products derived from the three- and four-membered rings would be the common amino acids, aspartic acid and glutamic acid, respectively. This hint of specific property of 1-amino*cyclo*pentane-1-carboxylic acid led to the synthesis and testing of α-aminoadipic acid which, however, proved to be ineffective.

Other obvious transformation products of CB 1639 are *cyclo*pentylamine, formed by the loss of carbon dioxide, and *cyclo*pent-1-ene-1-carboxylic acid, formed by the loss of ammonia. The amine proved to be surprisingly toxic (Table 5) but at a tolerated dose it had no effect on tumour growth. *cyclo*Pent-1-ene-1-carboxylic acid was also quite toxic when administered in water but it was well tolerated when given in aqueous sodium bicarbonate. The acid had no effect on tumour growth. It was felt that the stomach ulceration caused by CB 1639 might be associated with the local formation of this unsaturated acid since such irritation is not uncommon with unsaturated compounds.[11] Two other compounds which should be readily converted into the unsaturated acid are 1-hydroxy*cyclo*pentane-2-carboxylic acid (an isostere of CB 1639) and 1-amino*cyclo*pentane-2-carboxylic acid, this β-amino-acid loses ammonia even more readily than the tertiary α-amino-acid. The hydroxy-acid (CB 1693) was quite toxic and showed tumour growth inhibitory activity of a low order. 1-Amino*cyclo*-pentane-2-carboxylic acid (CB 1713) showed no appreciable anti-tumour activity but liver congestion and adhesions were observed in treated animals. Recently it has been shown that the amino-acid (CB 1713) which was submitted for test was the *trans*-isomer.[12]

Table 6 lists some miscellaneous compounds examined in the course of this work. None of these substances caused inhibition of the growth of the transplanted tumour, but there was evidence that several of the hydantoins caused retardation of growth in normal rats.

As the work progressed it became apparent that only compounds closely related to 1-amino*cyclo*pentane-1-carboxylic acid were effective as tumour growth inhibitors

and in order to obtain information about the mode of action a more detailed study of the effects produced by this compound was undertaken.

Pharmacological investigations of 1-*amino*cyclo*pentane*-1-*carboxylic acid* (*CB* 1639)

(a) *Haematological effects.* In preliminary haematological tests a single dose of 750 mg/kg of CB 1639 dissolved in water was administered by intraperitoneal injection to 4 male rats. This dose proved to be toxic as 3 rats died within 9 days of the injection. The weight and blood response curves of the survivor together with those of one of the animals which died on the ninth day after treatment are shown in Fig. 1

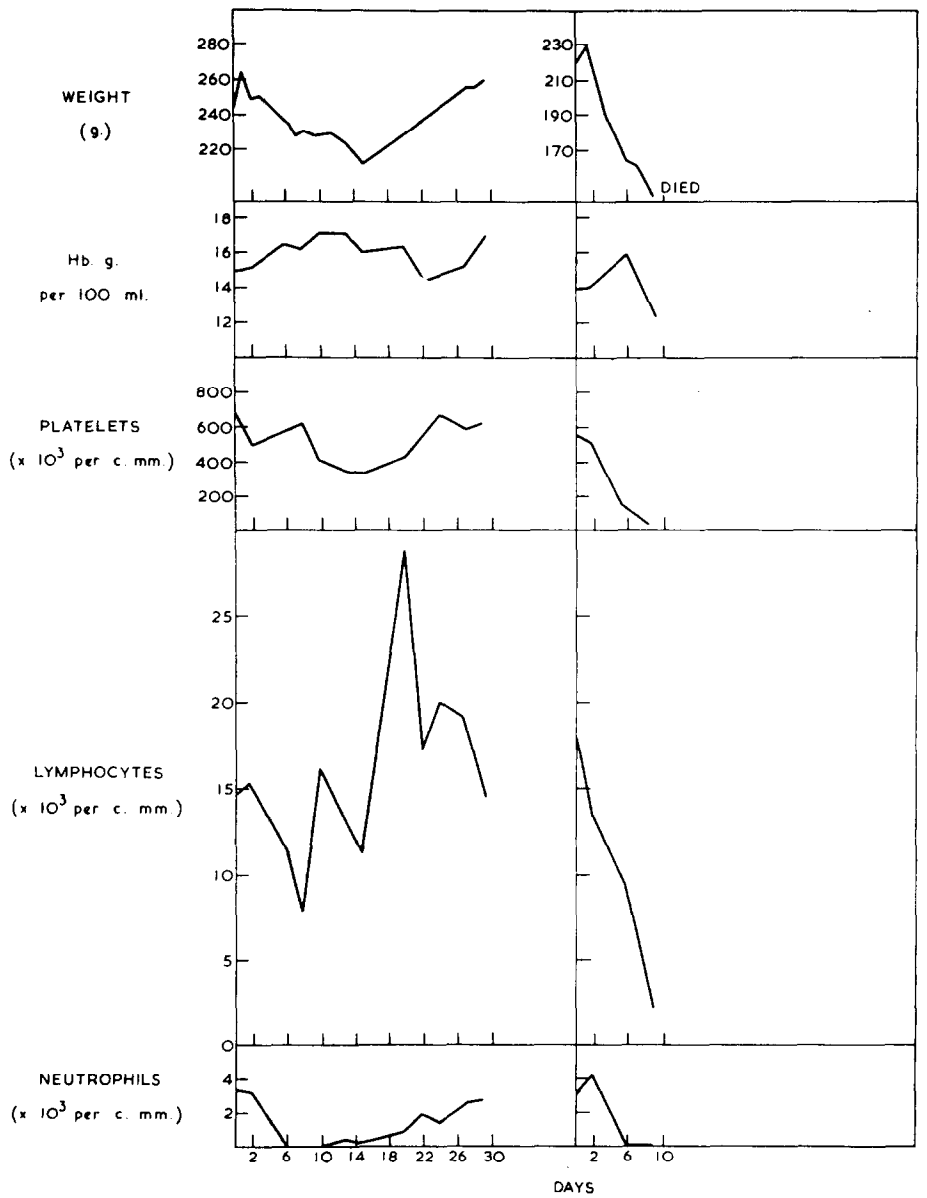


FIG. 1. Effect of CB 1639 (750 mg/kg i.p.) on the weight and blood of male rats.

The greatest effect is seen to be on the neutrophils which in both cases had completely disappeared from the circulation by the sixth day. In the case of the survivor small numbers of neutrophils began to reappear in the peripheral blood on the twelfth day and the numbers gradually increased until normal values were again attained about 30 days after the original injection. In the 3 animals that died no reappearance of neutrophils was observed, and the other blood elements all fell precipitously until death ensued.

At post-mortem of the rat dying at 9 days, only slight pathological changes related to the thrombocytopenia were observed. Superficial lymph nodes showed only minimal evidence of haemolymph change and there were a few small petechial haemorrhages in the skin. The adrenal glands were very dark and haemorrhagic. The main cause of death, however, was probably haemorrhage in the stomach which was filled with fresh blood and showed inflammation and haemorrhage in the pyloric portion. The thymus was small; heart and lungs appeared normal.

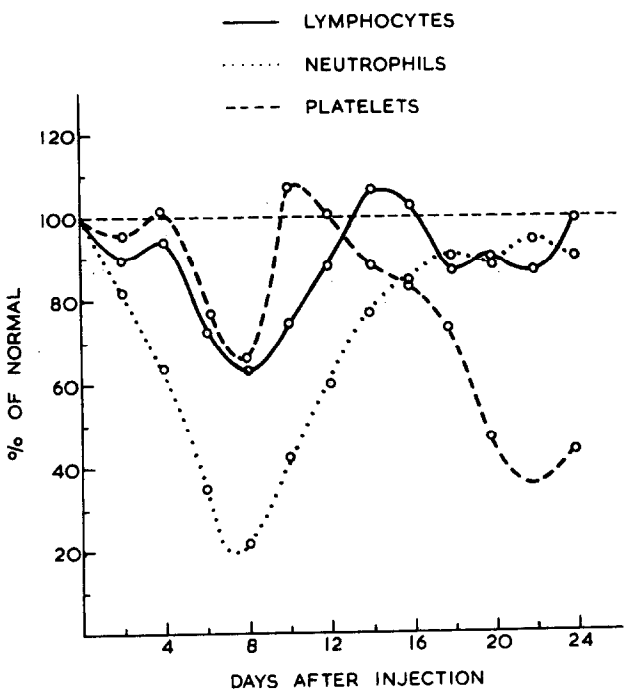


FIG. 2. Blood response pattern in male rats to CB 1639 (200 mg/kg i.p.).

Following this experiment, blood counts were carried out on 4 male rats treated with a non-lethal dose (200 mg/kg intraperitoneally). This dose caused some weight loss for a few days after the injection ($L_1 = 35$ g) but no other obvious detrimental effects. Figure 2 shows the percentage change from normal for lymphocytes, neutrophils, and platelets in the circulating blood. The curve represents the mean values from 4 rats. Again the main effect is on neutrophils which fall to 20 per cent of their normal value in about 7 days after the single injection of CB 1639. Recovery is then steady and normal values are regained after a further 8 to 10 days.

(b) *Growth inhibition and toxicity.* The most marked physiological effect of 1-amino*cyclo*pentane-1-carboxylic acid (CB 1639) is a prolonged inhibition of growth. This appears to be largely, though not entirely, due to loss of appetite and reduced food and water intake. Figure 3 shows the effect of CB 1639 (375 mg/kg administered intraperitoneally) on the weight of 6 female rats maintained on a "hard" rat cake diet and on nine fed a "soft mash" diet. On the rat cake diet all animals began to lose weight rapidly following the injection. One animal started to regain weight after 6 days and at post-mortem examination after 21 days showed no sign of stomach ulcers. The other 5 rats continued to lose weight steadily until death occurred 16–17 days after the injection. The rats which died all showed areas of haemorrhage and ulceration in the stomach. On the other hand, of the 9 animals fed the soft mash diet only one

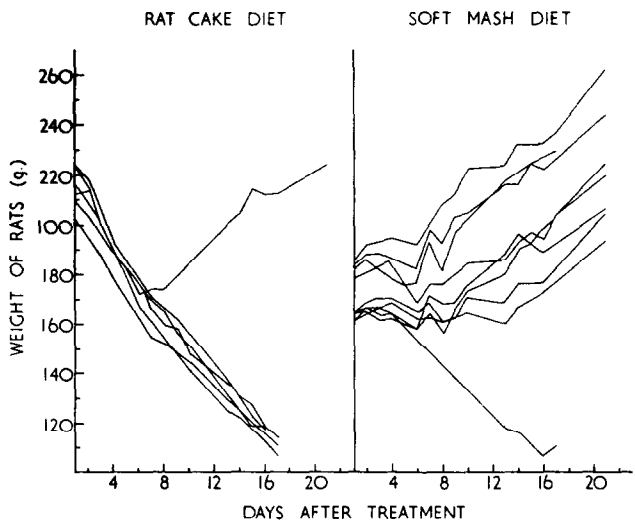


FIG. 3. Effect of CB 1639 (375 mg/kg i.p.) on the weight of female rats maintained on "rat cake" and "soft mash" diets.

showed a prolonged weight loss and stomach lesions. The other 8 rats showed relatively little weight loss and when examined at post-mortem after about 20 days they were free from stomach ulcers.

Some effects of the nature of the food offered on the quantities consumed are illustrated in Fig. 4. This shows the weight of food eaten by 2 male rats injected with CB 1639. A moderate amount of semi-soft food (bread) was eaten, but when this was replaced by "hard" dog biscuit, which is eaten readily by normal rats, only a small amount was consumed on the first day and it was refused on the next day. The soft mash diet given on the following 3 days was eaten in quantity but the dog biscuit was again refused when offered during the next 2 days. Subsequently both "mash" and bread were accepted. It is known that controls within the central nervous system, particularly within the hypothalamus are essential for the normal regulation of spontaneous food and water intake and some of the effects of 1-amino*cyclo*pentane-carboxylic acid might be due to a direct action on the hypothalamus. However, some

experiments carried out by Dr. D. G. Montemurro failed to demonstrate histologically that the effects of CB 1639 were caused by any primary cytotoxic action of the drug on the neurones of the lateral hypothalamic and medial subthalmic areas of the pituitary gland.

Although at first sight it may appear from these observations that the hard or soft nature of the food may be of primary importance in determining the toxicity of CB 1639 this simplified view is not supported by an experiment in which the rats were

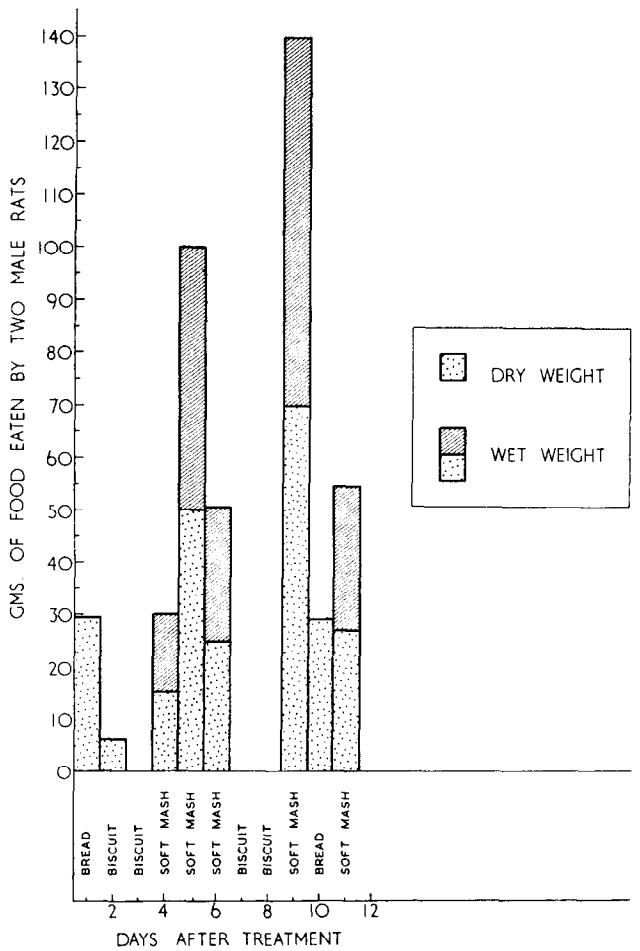


FIG. 4. Effects of the nature of the food offered on the quantities consumed by rats treated with CB 1639 (375 mg/kg i.p).

fed the rat cake in a powdered form made into a "mash" with water. The weight loss and survivals of the animals on this diet did not differ from that of those fed rat cake in its normal "hard" form.

Figure 5 shows the survival times of female rats treated with CB 1639 (375 mg/kg) maintained on "semi-soft" diets containing 5, 10 and 20 per cent protein in the form of casein. The lowest survival time and earliest deaths occurred in the animals on the lowest (5 per cent) protein diet whilst the best survival was obtained on the highest

(20 per cent) protein diet, although this rate is not nearly as good as can be obtained in animals receiving the same dose of CB 1639 and maintained on the "soft mash" diet (Fig. 3)

To investigate the question of what constituent of the "soft mash" diet might be mainly responsible for its beneficial effect comparative experiments were carried out

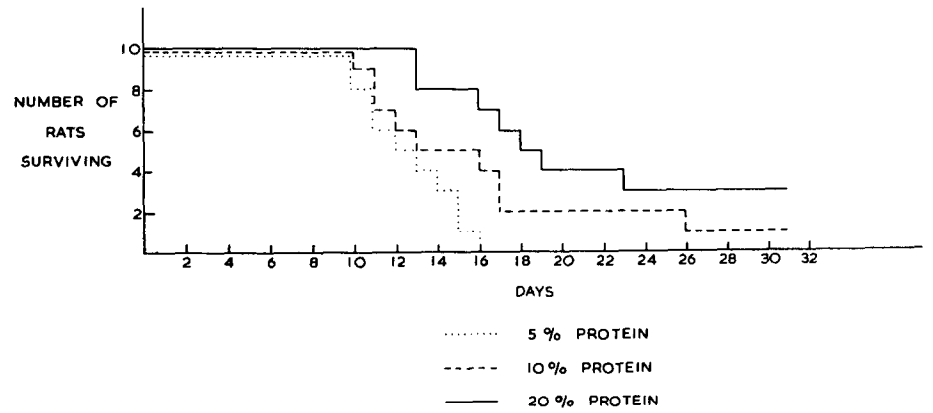


FIG. 5. Survival of female rats treated with CB 1639 (375 mg/kg i.p.) maintained on 5, 10, and 20 per cent protein diets.

with diets of "soft mash", "soft mash" without milk powder, water-soaked bread alone, and water-soaked bread with the addition of milk powder. The results are given in Fig. 6. These were carried out on male rats using a dose of 375 mg/kg. The survival on the soft mash diet is not as good as with female rats treated with the same dose of CB 1639 (Fig. 3) but 5 out of the 10 animals survived on this diet whils

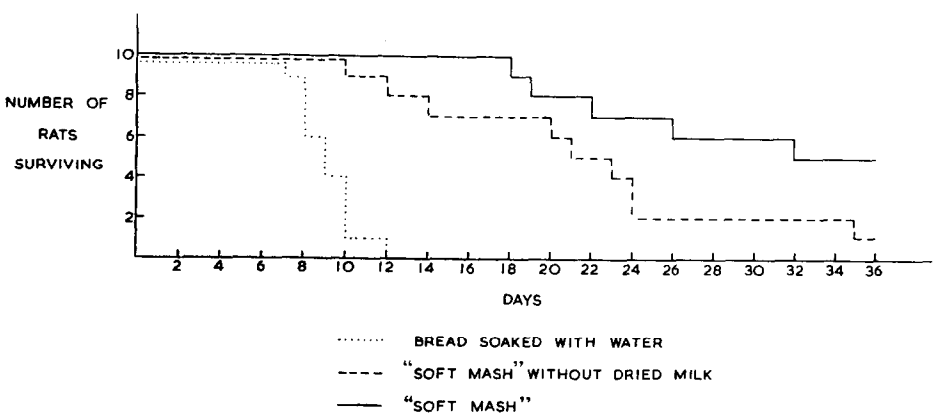


FIG. 6. Survival of male rats treated with CB 1639 (375 mg/kg i.p.) maintained on diets of bread soaked with water, "soft mash" without dried milk, and "soft mash".

there was only one survivor in the group receiving the mash without the addition of milk powder. The bread soaked with water proved to be a very poor diet as all the rats died within 7 to 12 days of the injection of CB 1639.

All rats dying in these groups showed signs of stomach lesions ranging in severity from small haemorrhages to large ulcers. The protective effect of the diet may be

mainly concerned in modifying the severity of these lesions. Obviously a high protein content is of considerable value, but the mere protein content is not the determining factor. It would seem that a "gastric ulcer diet" of soft consistency and a high milk content offers the best protection.

The course of development of stomach lesions in rats receiving CB 1639 is being investigated by pathological and histological examination of the stomachs of rats at frequent intervals after a single intraperitoneal dose of the compound. These results will be published elsewhere.

DISCUSSION

Of all the compounds tested for tumour inhibitory activity only CB 1639 and its ester and peptide derivative showed marked activity. Inhibition of the growth of the Walker carcinoma 256 and some inhibition of the growth of the transplantable mouse sarcoma 180 and of a transplantable mammary tumour in C$^-$ Bagg albino mice was demonstrated.

These results are similar to those obtained by Ross *et al.*[13] using the mouse tumours, sarcoma 180, carcinoma 755, and the leukaemia L 1210. Only CB 1639 and its simple derivatives were effective inhibitors and of these tumours the carcinoma 755 showed the greatest response. Marked tumour inhibition is, however, only obtained with doses which cause a considerable body weight loss to the animal.

The tumour growth inhibition, however, cannot be attributed entirely to the general toxic action or body growth inhibitory action of the compound. For instance, *cyclo*-pentylamine (CB 1689) is more toxic than CB 1639 but exhibits no inhibitory action on the Walker tumour.

The influence of nutritional status on the growth of Walker rat carcinoma 256 has been investigated by Walpole[14] who concludes that while compounds which interfere with body growth may be expected to inhibit tumour growth as a result of their general "toxic" action, this inhibition is unlikely to exceed that which would result from underfeeding at a comparable level. Underfeeding at the level required to prevent any increase in mean gross weight of the rat inhibits tumour growth by about 40 per cent. Reduction of food intake to only 3 g per rat per day, which resulted in about 16 per cent loss of body weight over 14 days led to about 60 per cent inhibition of tumour growth—in our method of assessing tumour growth inhibition this would correspond to a C/T ratio of about 2·5. Elson and Haddow[15] and Elson[16] found that maintaining rats on a 5 per cent protein diet made relatively little difference to the weights of Walker tumour compared with controls maintained on a 20 per cent protein diet.

It seems very unlikely, therefore, that C/T ratios as high as 30 obtained with CB 1639 (Table 1) could be attributable solely to a general toxic action causing inhibition of body growth and food consumption.

Like many other tumour-growth inhibitory agents CB 1639 also has a considerable effect on haemopoiesis. The main effect in the case of CB 1639 is a fall in the number of circulating neutrophilis and in this respect it resembles the effect of Myleran and dimethylmyleran[17] or perhaps more closely that of thioguanine.[18] The fall in neutrophils seems to be accompanied by a less drastic fall in the blood platelets than occurs with other compounds so that, but for its toxic properties, causing weight loss and

liability to stomach ulcers, it might have been expected to have shown some promise in the treatment of chronic myeloid leukaemia.

Some clinical trials of CB 1639 in cases of a variety of advanced malignant disease have been very briefly reported.[13, 19] Virtually no beneficial effects were observed.

The mode of action of CB 1639 and the specificity of the five-membered ring structure remain unelucidated. Microbiological experiments have not indicated that the compound is an antagonist of any of the usual amino acids.[20] The formation *in situ* of α-aminoadipic acid by the process described above stands as an attractive possibility. Despite the inactivity of the injected aminodicarboxylic acid, this might still be the effective compound since such an acid would not readily penetrate tumour cells—normal transport mechanisms not operating.[21] If formed within the cell, aminoadipic acid would persist and possibly interfere with lysine metabolism. It would seem well worth while examining some other aminoadipic acid derivatives, such as a range of diesters, which could diffuse into the cells and there release the amino acid.

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