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Cycloaliphatic Amino Acids in Cancer Chemotherapy

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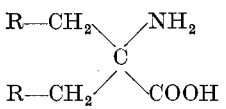
Very early in the anticancer testing programme conducted by the Cancer Chemotherapy National Service Center, Professor C. I. Noll, of the Pennsylvania State University, submitted a considerable number of compounds for antitumour evaluation. Among these, solely by chance, was the amino acid, 1-aminocyclopentanecarboxylic acid,

which was assigned the accession number NSC–1026 (NSC referring to National Service Center). Professor Noll had become interested in this compound and in α-substituted α-amino acids generally, from the viewpoint of the physical properties of these unusual amino acids and the hydrolytic reaction rates of the corresponding precursor hydantoins.

Simultaneously, the Chester Beatty Research Institute synthesized 1-aminocyclopentanecarboxylic acid (CB 1639) for trial as a potential anticancer agent as an outcome of their interest in α-substituted amino acids:



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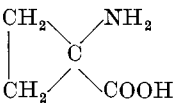
‡ Chester Beatty Research Institute, Royal Cancer Hospital, Fulham Road, London, S.W.3, England.

as growth inhibitors. As a logical extension, the two alkyl chains were joined to provide first the cyclopropane amino acid



and then other cycloaliphatic acids including the 1-aminocyclopentanecarboxylic acid. Thus, the synthesis and antitumour evaluation of CB 1639 was arrived at by the Chester Beatty Research Institute on a rational basis and completely independently of the Cancer Chemotherapy National Service Center programme.

NSC–1026 had been prepared after the turn of the century by Zelinskii[1] using the now classical hydantoin synthesis. Bucherer and Lieb[2] described a modification of this method in which the test compound is warmed in dilute alcoholic solution with potassium or sodium cyanide and ammonium carbonate, the hydantoin usually separating in an almost pure state from the reaction mixture on cooling. The hydantoins so prepared may be hydrolyzed to the expected amino acid by a variety of procedures, chiefly, however, by heating in solutions of saturated barium hydroxide or 60 per cent aqueous sulphuric acid.

In the course of Professor Noll's investigations, it was found that the acid ionization constant, pK_2, of 1-aminocyclopentanecarboxylic acid differed only slightly from that of an 'open-chain' analogue, 2-amino-2-methylbutyric acid[3] or from that of α-aminoisobutyric acid.

Formal titrations designed to test the steric effect imposed on 1-aminocyclopentanecarboxylic acid by the structural features of the molecule, demonstrated that neither this compound (NSC–1026) nor α-aminoisobutyric acid produced any indication of maxima at the expected neutralization point.[3] This phenomenon was found to hold true with α-amino-α-phenylpropionic acid.[3] It is logical to assume a decreased reactivity of the amine function due to the amine and carboxylate functional groups being attached directly to one carbon atom of the cyclopentane ring or to the presence of an alkyl or aryl group in the α-position.

A few other properties of NSC–1026 may be of interest. The

compound has a melting point of 328–329° after drying for three hours at 120°. Its solubility in water is about 5 g per 100 ml. The mobility of NSC–1026 in paper chromatography is strikingly different from that of the naturally occurring amino acids. Leucine has the most similar R_f value but the two are readily distinguishable.[4] NSC–1026 has a sweet taste, a fact first noted by Zelinskii.[1]

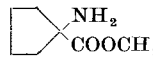
The cycloaliphatic amino acids which have been prepared chiefly by Noll[5] and by the Chester Beatty Institute[6] are given in Table I.

In addition to the compounds listed in Table I, glycylpeptides of 1-aminocyclopentanecarboxylic acid and cyclopropylalanine were synthesized and tested by the Chester Beatty group.

Antitumour activity of all the compounds listed in Tables I and II, as far as can at present be determined, is confined exclusively to 1-aminocyclopentanecarboxylic acid, its simple esters, its hydroxylamino derivative (NSC–21436) and its glycylpeptides. The test system employed by the Chester Beatty group was the Walker rat carcinosarcoma 256. The compounds designated by NSC accession numbers were evaluated in the Cancer Chemotherapy National Service Center primary screening test systems, composed of the sarcoma 180, carcinoma 755 and leukemia L–1210 mouse tumours. For the most part, NSC–1026 and its simple esters were most effective in the carcinoma 755 test, significant inhibition of tumour growth being observed without undue weight loss. The Chester Beatty group reports that the glycylpeptide CB–1668 was not well tolerated in the rat at a dose level of 50 mg per rat; at the 25-mg level both CB–1668 and 1659 produced marked diminution of tumour growth and were well tolerated.

It has been thoroughly established, at least with respect to the animal test systems used, that substitution on the cyclopentane ring destroys activity and that the *N*-derivatives, with the exception of the hydroxylamino and *N*-glycyl peptides, do not permit activity; even as simple a derivative as the copper salt effectively prevents the appearance of biological activity in the rodent tumour systems studied to date. It is especially noteworthy, however, that antitumour activity is at least retained by low molecular-weight peptides.

Table I. NSC–1026 and related compounds

NSC no.	CB no.	Structure	Name	Source[a]
1026	1639		1-Aminocyclopentanecarboxylic acid	A, B, E
26981	1704		Methyl 1-aminocyclopentanecarboxylate	A
9877	1691		Ethyl 1-aminocyclopentanecarboxylate	B, D
26982	—		*n*-Propyl 1-aminocyclopentanecarboxylate hydrochloride	A, B
26983	1708		Isopropyl 1-aminocyclopentanecarboxylate	A
26985	—		*n*-Butyl 1-aminocyclopentanecarboxylate	A
26984	—		Isobutyl 1-aminocyclopentanecarboxylate hydrochloride	A
2822	—		Cupric 1-aminocyclopentanecarboxylate	A
—	1705		1-Aminocyclopentanecarboxamide	B

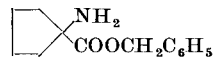
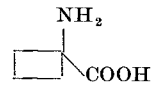
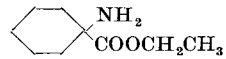
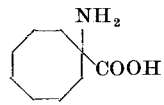
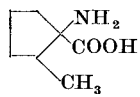
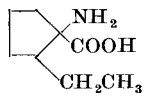
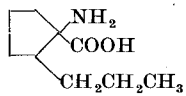
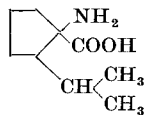
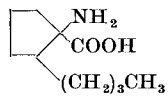
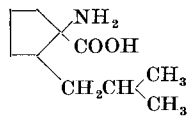
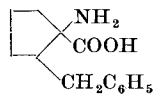
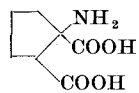
26986	—		Benzyl 1-aminocyclopentanecarboxylate	A
—	1703		1-Aminocyclopropanecarboxylic acid	B
—	1700		1-Aminocyclobutanecarboxylic acid	B
9059	1641		1-Aminocyclohexanecarboxylic acid	A, B, E
9878	—		Ethyl 1-aminocyclohexanecarboxylate	D
22849	1692		1-Aminocycloheptanecarboxylic acid	A, B
22851	1714		1-Aminocyclooctanecarboxylic acid	A, B
32823	- -		1-Amino-2-methylcyclopentanecarboxylic acid	A
2825	—		1-Amino-2-ethylcyclopentanecarboxylic acid	A

Table I. NSC–1026 and related compounds—*cont.*

NSC no.	CB no.	Structure	Name	Source[a]
2830	—		1-Amino-2-*n*-propylcyclopentanecarboxylic acid	A
2831	—		1-Amino-2-isopropylcyclopentanecarboxylic acid	A
2836	—		1-Amino-2-*n*-butylcyclopentanecarboxylic acid	A
2837	—		1-Amino-2-isobutylcyclopentanecarboxylic acid	A
2845	—		1-Amino-2-benzylcyclopentanecarboxylic acid	A
—	1701		1-Amino-1,2-cyclopentanedicarboxylic acid	B

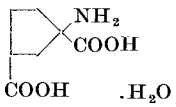
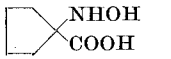
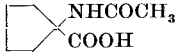
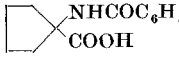
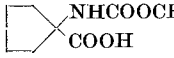
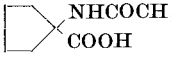
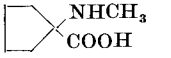
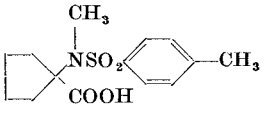
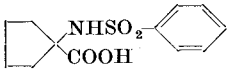
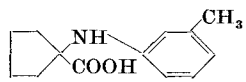
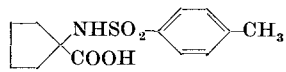
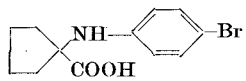
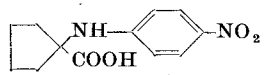
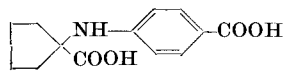
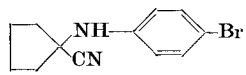
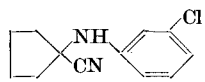
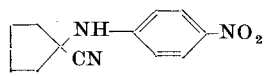
27386	1712		*trans*-1-Amino-1,3-cyclopentanedicarboxylic acid monohydrate	B, D
21436	—		1-(Hydroxylamino)cyclopentanecarboxylic acid	F
22847	1709		1-Acetamidocyclopentanecarboxylic acid	A
33394	1702		DL-1-Ureidocyclopentanecarboxylic acid	B, C
22854	—		1-Benzamidocyclopentanecarboxylic acid	A
22848	—		Methyl 1-carboxycyclopentanecarbamate	A
—	1732		1-(2-Chloroacetamido)cyclopentane-carboxylic acid	B
—	1728		1-Methylaminocyclopentanecarboxylic acid	B
—	1737		1-(*N*-Methyl-*p*-toluenesulphonamido)-cyclopentanecarboxylic acid	B
22853	—		1-(Benzenesulphonamido)cyclopentane-carboxylic acid	B

Table I. NSC–1026 and related compounds—*cont.*

NSC no.	CB no.	Structure	Name	Source[a]
22855	—		1-(*m*-Toluidino)cyclopentanecarboxylic acid	A
32846	1736		1-(*p*-Toluenesulphonamido)cyclopentanecarboxylic acid	A
43104	—		1-(*p*-Bromoanilino)cyclopentanecarboxylic acid	A
43105	—		1-(*p*-Nitroanilino)cyclopentanecarboxylic acid	A
43109	—		1-(*p*-Carboxyanilino)cyclopentanecarboxylic acid	A
43101	—		1-(*p*-Bromoanilino)cyclopentanecarbonitrile	A
43102	—		1-(*m*-Chloroanilino)cyclopentanecarbonitrile	A
43103	—		1-(*p*-Nitroanilino)cyclopentanecarbonitrile	A

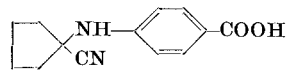
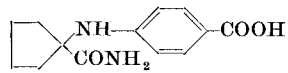
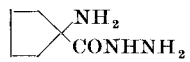
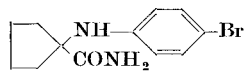
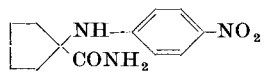
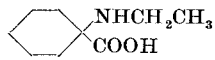
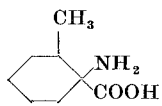
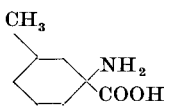
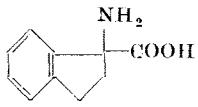
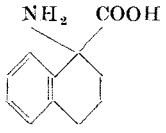
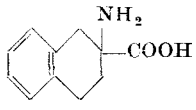
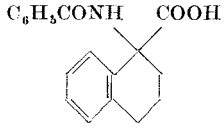
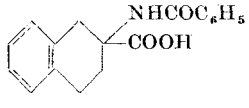
43108	—		*p*-(1-Cyanocyclopentylamino)benzoic acid	A
43110	—		*p*-(1-Carbamoylcyclopentylamino)benzoic acid	A
42315	—		1-Amino-1-cyclopentanecarboxylic acid hydrazide	C
43106	—		1-(*p*-Bromoanilino)cyclopentanecarboxamide	A
43107	—		1-(*p*-Nitroanilino)cyclopentanecarboxamide	A
20894	—		1-Methylaminocyclohexanecarboxylic acid	E
20893	—		1-Ethylaminocyclohexanecarboxylic acid	E
39063	1645		DL-1-Amino-2-methylcyclohexanecarboxylic acid	B, C
—	1638		1-Amino-3-methylcyclohexanecarboxylic acid	B

Table I. NSC–1026 and related compounds—*cont.*

NSC no.	CB no.	Structure	Name	Source[a]
32833	1696		DL-1-Amino-1-indanecarboxylic acid	A, B, C
39062	1643		DL-1-Amino-1,2,3,4-tetrahydro-1-naphthoic acid	B, C
37016	1647		DL-2-Amino-1,2,3,4-tetrahydro-2-naphthoic acid	B, C
—	—		1-Benzamido-1,2,3,4-tetrahydro-1-naphthoic acid	B
—	—		2-Benzamido-1,2,3,4-tetrahydro-2-naphthoic acid	B

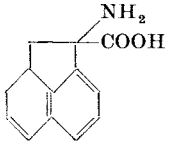
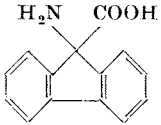
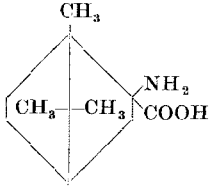
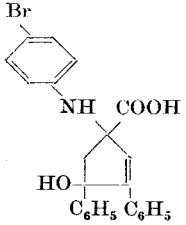
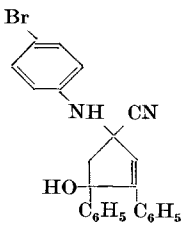
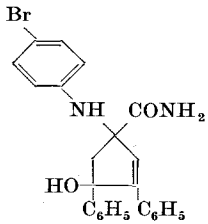
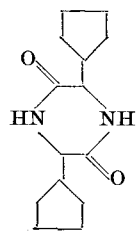
43246	—		DL-1-Amino-1-acenaphthenecarboxylic acid	C
32848	—		9-Aminofluorene-9-carboxylic acid	A
32841	—		2-Amino-2-bornanecarboxylic acid	A
43112	—		1-(*p*-Bromoanilino)-4-hydroxy-3,4-diphenyl-2-cyclopentene-1-carboxylic acid	A

Table I. NSC–1026 and related compounds—*cont.*

NSC no.	CB no.	Structure	Name	Source[a]
43111	—		1-(*p*-Bromoanilino)-4-hydroxy-3,4-diphenyl-2-cyclopentene-1-carbonitrile	A
43113	—		1-(*p*-Bromoanilino)-4-hydroxy-3,4-diphenyl-2-cyclopentene-1-carboxamide	A
32851	—		3,6-Dicyclopentyl-2,5-piperazinedione	A

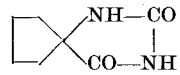
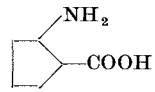
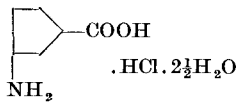
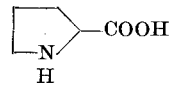
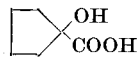
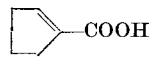
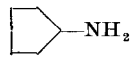
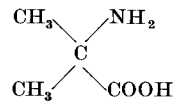
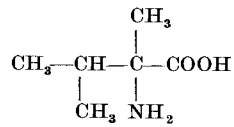
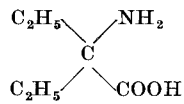
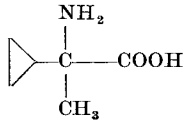
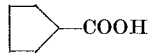
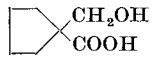
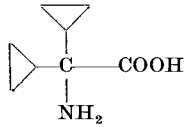
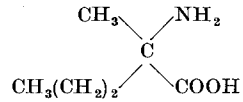
1024	1683		1,3-Diazaspiro[4,4]nonane-2,4-dione	A, B
—	1713		2-Aminocyclopentanecarboxylic acid	B
27385	—		*cis*-3-Aminocyclopentanecarboxylic acid, hydrochloride, $2\frac{1}{2}$ hydrate	D
46703	1707		L-Proline	B
—	1693		1-Hydroxycyclopentanecarboxylic acid	B
44968	1684		Cyclopentene-1-carboxylic acid	B
—	1689		Cyclopentylamine	B
16590	1637		2-Methylalanine	B
23276	—		2-Amino-2,3-dimethylbutyric acid	A

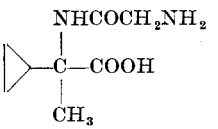
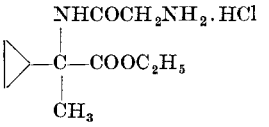
Table 1. NSC–1026 and related compounds—*cont.*

NSC no.	CB no.	Structure	Name	Source[a]
23275	1686		2-Amino-2-ethylbutyric acid	A
39061	1653		DL-α-Amino-α-methylcyclopropane-acetic acid	C
—	1725		Cyclopentanecarboxylic acid	B
—	1722		1-(Hydroxymethyl)cyclopentanecarboxylic acid	B
32354	—		2,2-Dicyclopropylglycine	C
23277	1685		2-Methylnorvaline	B

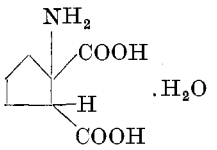
[a] A: C. I. Noll, Pennsylvania State University
B: Chester Beatty Research Institute, Alexander Haddow, Director
C: Midwest Research Institute under Contract No. SA-43-ph-2394 with the Cancer Chemotherapy National Service Center
D: Leslie Hellerman, Johns Hopkins University
E: L. Berlinguet, Laval University, Quebec, Canada
F: J. Andrako, J. D. Smith and W. H. Hartung, Medical College of Virginia, Richmond, Virginia

The only exception known to us is that 1-aminocyclohexanecarboxylic acid has shown biological activity in the Novikoff hepatoma system.[7]

Table II.

CB no.	Structure	Name
1668		*N*-[(1-Amino-1-cyclopentyl)carbonyl]-glycine
1659		1-(2-Aminoacetamido)cyclopentanecarboxylic acid
1676		α-(2-Aminoacetamido)-α-methylcyclopropaneacetic acid
1706		Ethyl α-(2-Aminoacetamido)-α-methylcyclopropaneacetate hydrochloride

The compound assigned the accession number NSC–27386 was prepared both by Professor Leslie Hellerman, Johns Hopkins University[8] and the Chester Beatty Research Institute. Dr. Hellerman submitted the *trans* diastereoisomeric pair, 1-amino-*trans*-1,3-cyclopentanedicarboxylic acid, monohydrate (NSC–27386)



The *trans* configuration, although not unequivocally proved, is regarded as the most likely conformation of the substance. With respect to the two lower members of this cycloaliphatic series, 1-aminocyclopropanecarboxylic acid was first made by Ingold[9]

and reinvestigated by Rinderknecht and Niemann.[10] The latter authors tested this compound with respect to its ability to function as a precursor, or antagonist, of methionine and threonine using γ-amino-γ-carboxypropyltrimethylammonium iodide as a methyl donor. All tests were negative.

An interesting variant of NSC–1026 is the corresponding hydroxyamino acid, 1-(hydroxylamino)cyclopentanecarboxylic acid (NSC–21436).[11] This compound has demonstrated biological activity in the leukemia L–1210 animal tumour system employed by the Cancer Chemotherapy National Service Center; its biological activity does not appear to differ from other simple derivatives of NSC–1026.

1-Aminocyclopentanecarboxylic acid has been found to occur in nature. Burroughs[12] has isolated the free amino acid from perry pears and states that it appears during the last three or four weeks of ripening on the tree. The occurrence of this acid in nature has also been noted by Virtanen.[13]

The biological reactions of NSC–1026 in the experimental rodent have been summarized by Connors *et al.*[14] At the present time, no explanation of the mode of activity of this compound is possible.

An alternative postulation arises from the observation that NSC–1026 forms stable metal salts and could be considered to remove trace metals by chelation or salt formation.

It would be reasonable to look for antagonism of NSC–1026 toward essential amino acids. However, such antagonism has not as yet been noted in the limited experimentation carried out at the Chester Beatty Institute and at the Midwest Research Institute.[15] Workers at the latter Institute found that NSC–1026 did not interfere with the utilization of leucine, isoleucine or valine using *Streptococcus faecalis* R–NRRL as the test organism. The structural specificity of some amino acid antagonists has been investigated by Shive *et al.*[16] who made a study of the minimal size of the grouping necessary to induce phenylalanine antagonism in β-substituted alanines which have the β-carbon in the same plane as the adjacent carbons of the substituent group. It is evident that 1-aminocyclopentanecarboxylic acid must be studied further to elucidate its mechanism of biological action.

Following the appearance of reproducible biological activity in one of the Cancer Chemotherapy National Service Center test

systems by NSC–1026, the compound was subjected to preclinical pharmacological trial.

Pharmacological Studies on NSC–1026

Pharmacological studies on 1-aminocyclopentane-1-carboxylic acid (NSC–1026) were carried out by the Food and Drug Administration Laboratory, Washington, D.C., and at The Christ Hospital Institute for Medical Research, Cincinnati, Ohio. The studies were based on a protocol recommended by the Cancer Chemotherapy National Service Center as follows.

Mice and rats. Acute toxicity was determined by both the oral and the parenteral routes. Dose-ranging studies were performed on groups smaller than ten animals to determine the LD_{50} and the MED. All animals were dosed on the same day following an overnight fast. The body weight range was kept as narrow as possible. All side effects including the nature and time sequence, recovery rate of survivors, time of death in non-survivors, and any other toxic or pharmacological effects were recorded. The animals were observed until resumption of normal behaviour and normal rate of food intake, with a minimum of 14 days and a maximum of 28 days.

Dogs and monkeys. Two animals (1 male and 1 female) which had been fasted overnight were dosed at each of 3 or 4 dose levels. A fixed log interval such that minimal effects were produced by the lowest dose, and death of at least one animal occurred at the highest dose, was used. All observations on side effects, particularly to the respiratory, cardiovascular, and nervous systems, were recorded. Animals were observed until the resumption of normal behaviour and normal food intake for at least 14 days and no longer than 28 days.

Repeated dose toxicity. Ten rats per group plus a control group and two dogs, one male and one female (no control group required for dogs), were used for each dose level. The drug was administered for four weeks using the route and dosage prescribed by the CCNSC. Weekly records of mean body weights by groups and mean daily food intakes by groups (these were recorded individually for dogs and monkeys) based on weekly food consumption were kept. At the end of the dosage period, half the

surviving animals of each sex in each dosage group and half the control animals were sacrificed and examined for gross pathology and histopathology. The remaining animals were placed on normal diets until normal behaviour and normal food intake returned, or until four weeks passed, whichever came first. These animals were sacrificed and examined for gross and microscopic pathology.

Experimental animals were kept under a quarantine period for two weeks prior to testing. During this time, appropriate immunizations for distemper and tests for haemoglobin, packed cell volume, total white count, and differential cell count, were performed at least twice. Also, before the dosage was started, a normal range of liver function and kidney function was demonstrated. Post-mortem examinations on all deaths which occurred were done unless the post-mortem changes were too advanced.

Results

Table III. Single dose toxicity

Species	Route	LD_{50}	Dose, mg/kg	Mortality	Dose, mg/kg	Mortality	Dose, mg/kg
Mouse	Oral	309	199	0/10	1000	9/10	91
Rat	Oral	290	159	0/6	316	6/6	163
Rat	i.v.	340	125	0/2	398	8/10	173
Guinea pig	Oral	140	79	0/4	159	4/4	69
Dog	Oral	*ca.* 300					
Dog	i.v.	*ca.* 300					

Mice. Drug dose levels spaced at 1/10 log dose intervals from 199·5 mg/kg to 1259 mg/kg were administered by stomach tube to young adult mice. The toxic symptoms displayed were delayed anorexia, diarrhoea, severe malaise, and delayed deaths occurring from 7 to 21 days after treatment. Histological examination of two male mice receiving 1000 mg/kg which were sacrificed on the sixth day showed cloudy swelling to marked fatty degeneration in the liver, tubular dilatation in the kidneys, thymic cortical atrophy in one animal, slight bone marrow atrophy and congestion. The animals failed to eat after the administration

of the drug. Histopathology in some of the treated animals included myocardial degeneration, renal tubular dilation, atrophy of the pancreas and bone marrow, and slight demyelinization in the central nervous system. Atrophy, cloudy swelling and fatty degeneration of the hepatic cells were more marked in the treated animals than in the starved control mice.[17]

Rats. The drug was given to rats both orally and intravenously with doses ranging from 15·6 mg/kg to 1000 mg/kg. The animals showed little effect during the initial 3 or 4 days after drug administration. However, this was followed by severe anorexia, watery stools, and delayed deaths of from 7 to 21 days (average of 14 days). Histopathology revealed that those animals given 500 mg/kg, intravenously, had atrophy of bone marrow, liver and pancreas, slight atrophy of the testes, adrenal cortical hypertrophy and focal myocardial degeneration. Rats given 1000 mg/kg, orally, showed similar effects. Guinea pigs reacted similarly at a dose range of 79·4 mg/kg to 251·2 mg/kg but the average day of death was day four.[17]

Cats. Two cats infused with 250, 350, or 500 mg/kg each, and observed from one to four hours showed a slight lowering of blood pressure and no effect on the electrocardiagram. Characteristic responses to histamine, ephedrine and acetycholine were not affected by infusion of the drug.[17]

Repeated Dose Toxicity

Rats. Feeding experiments. Groups of four rats each were fed doses of the drug at levels of 2000, 500, 250, and 125 parts per million for a four-week period. All rats given toxic doses showed an extreme weight loss occurring from day 11 to 17, and all deaths occurred during this period. Rats given 125 ppm for four weeks were alive at the end of the treatment period and the examined tissues revealed no abnormal pathology. Histopathological examination of those receiving toxic doses showed atrophy of bone marrow, liver and pancreas, adrenal congestion and marked erythroid shifts with myeloid/erythroid ratios of approximately 1 : 8.

In rats given repeated doses, the toxicity appeared to be cumulative and comparisons of the intravenous and oral routes were

quantitatively and qualitatively the same with respect to toxic effects.[17]

In order to determine whether there was any immediate histological change which might be cumulative and irreversible, resulting in delayed deaths of the animals, or whether the direct effects of this drug only occurred slowly, a test was performed on six young male rats. Each rat was injected once, intravenously, with 500 mg/kg of NSC–1026, and then two rats were sacrificed for histological examination at each interval of 6, 13 and 23 h after treatment. Two control rats were sacrificed at the same time as the 23-h post-treatment animals. No gross pathology was noted. Microscopic examination showed only very slight hepatic and pancreatic changes of undetermined significance which might be attributable to drug toxicity.[17]

Dogs. By a rough approximation, the single dose LD_{50} in dogs by the intravenous, as well as the oral route, was found to be 300 mg/kg. Repeated dose studies on dogs, at a dosage level of 1, 3·16, 10 and 31·6 mg/kg-day for thirty days by the oral route (capsule form) showed no gross pathology in the dogs receiving 10 mg/kg-day or less. The animals given 31·6 mg/kg daily had anorexia, a weight loss after two weeks, were moribund when sacrificed, emaciated, and had an empty gastrointestinal tract. One animal had bloody stools the day before autopsy. One animal had blood in the ileum and secum and one female had a large stomach ulcer and enlarged, darkened adrenals. Microscopic pathology revealed changes in these dogs which included atrophy of the bone marrow, liver, pancreas and testes, moderate damage to the kidneys and adrenals, slight demyelinization of the cerebellum, splenic haemosiderosis, and a myeloid/erythroid ratio of 5 : 1.[17]

Dogs succumbed to daily doses of 60 mg/kg or greater, injected intravenously, between day 8 and day 15 (average was day 12), and the toxicity of the drug was the same whether given once or twice daily. The toxic reactions of dogs to fatal doses included severe malaise, anorexia, tremors, haemoconcentration, and low-grade reticulocytopenias and thrombocytopenias. Necropsy findings indicated extensive petechial haemorrhages in the mucosa of the entire gastrointestinal tract and splenic atrophy.[18]

Monkeys. The drug was comparatively well tolerated in the

rhesus monkey when injected intravenously. With the exception of one animal, no monkey receiving 120 mg/kg daily exhibited significant toxic reactions. Monkeys given 240 mg/kg-day or greater for thirty days succumbed to the drug whether given once or twice daily. The toxic symptoms noted were diarrhoea, severe malaise, haemoconcentration, collapse of the peripheral circulation, and in some instances a low-grade leucopenia. Necropsy findings revealed petechial haemorrhages in the mucosa of the cardiac stomach, atrophy of the spleen, intussusception of the ileum, and haemorrhagic lesions in the ileum both above and below the obstruction. The monkeys also exhibited a slight erythroid depression. The average day of death for the animals receiving 240 mg/kg daily or greater was day 8 or day 9. In no case were the reactions as severe as those found in the dogs.[18]

Biochemical Reactions and other Tests

The most unusual action of NSC–1026 was the lack of immediate toxic symptoms followed by delayed toxicity resulting in death from lethal doses, sub-lethal doses producing prolonged anorexia. Deaths occurred on days 7 to 21 after administration of the drug. The histopathological changes that normally occur with delayed deaths were absent.

The concentration of the drug in the serum of the monkeys and dogs receiving repeated doses, orally, increased with dose. In all animals the concentration of NSC–1026 in serum fell rapidly after intravenous injection. Four hours, thereafter, the apparent level of the drug increased, in some cases, to reach a height at 8 h substantially greater than that level at $\frac{1}{2}$-h post treatment. This was followed by a very slow decline in drug levels, but was still substantial at 48 h. The material detected in the serum was NSC–1026 or a substance with essentially the same mobility, as shown by paper chromatographic studies.[18]

NSC–1026 also exhibited an unusual reaction on the blood glucose levels. Rats fed 40 mg/kg-day for three days showed a rise in the blood glucose level on days 4, 7 and 14 after initial treatment. The pair-fed controls showed a drop in the blood glucose level as compared with the control animals. Rats fed 6 mg/kg-day for four days had a weight decrease in the pituitary

glands and animals fed 60 mg/kg-day for four days had a weight decrease in the thyroid glands and a weight increase in the thymus glands.[17]

Results of Clinical Trials using NSC–1026

Following the accumulation of the necessary pharmacological data, NSC–1026 was obtained in a suitable pharmaceutical formulation for clinical trial under the aegis of the Cancer Chemotherapy National Service Center.

A group of Veterans Administration hospitals studied NSC–1026 in a series of 71 patients with a variety of far advanced malignancies.[19] The drug was given intravenously or orally in doses ranging from 10–150 mg/kg-day over 5–10 day periods.

Virtually no beneficial effects were observed in this series, although one patient with malignant melanoma did exhibit signs of objective and subjective improvement.

Toxic manifestations, consisting solely of anorexia, nausea and vomiting, were observed in some patients receiving doses of 60 mg/kg-day or greater. No deaths could be directly attributed to the drug.

Summary. 1-Aminocyclopentanecarboxylic acid was discovered to possess antitumour activity in experimental animals. This discovery was made simultaneously and independently by the Chester Beatty Research Institute and the Cancer Chemotherapy National Service Center. This biological activity appears to be restricted to the intact cyclopentane amino acid and certain derivatives and peptides of it. This compound was not efficacious in treatment of a variety of neoplastic growths in the human.

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