428. *The Relation between the Constitution of Arsenicals and their Action on Cell Division.*

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Since cacodylic acid is a mitotic poison some homologues and other readily accessible types of aliphatic arsenical were synthesised and their action on living cells was examined by a tissue-culture technique. Two main types of mitotic aberrations were found, dependent on whether the action was primarily on the spindle (" colchicinic effect ") or on the chromosomes.

IT has become customary to apply the term mitotic poison to any substance capable of bringing about aberrations of mitosis in animal or plant cells. Mitotic poisoning of mammalian cells has acquired practical significance in the search for chemotherapeutic agents capable of inhibiting malignant growth.

Dustin and his collaborators first directed attention to the profound disturbance of cell division which followed the injection of arsenicals into mice (Piton, *Arch. Int. Med. Exp.*, 1929, **5**, 355; Dustin and Piton, *Bull. Acad. Roy. Med. Belg.*, 1929, **9**, 26; Dustin and Grégoire, *Compt. rend. Soc. Biol.*, 1933, **114**, 195; *Bull. Acad. Roy. Med. Belg.*, 1933, **13**, 585). Cacodylic acid was the most potent of the compounds investigated [(others were arsenious oxide, methylarsonic acid, and aminophenylarsonic acid (atoxyl)] and it also stimulated mitosis in cells of the crypts of Lieberkühn and of transplanted tumours. The chromosomes of dividing cells were described as being clumped together in the middle of each cell and being surrounded by clear œdematous-looking cytoplasm.

Interest in the arsenicals became diverted by the discovery of the unique mitotic poisoning properties of the alkaloid colchicine. By employing the tissue-culture technique, Ludford (*Arch. Exp. Zellforsch.*, 1936, **18**, 411) showed that the cytological actions of colchicine and cacodylic acid were identical. Both substances arrested mitosis at metaphase by preventing the formation of the mitotic spindle.

Owing to the complexity of the colchicine molecule it seemed to us that, for investigating the relation between chemical constitution and mitotic poisoning, arsenicals would provide an ideal class of compound. In this investigation attention has been confined to the following: (i) methyl-, propyl-, butyl-, benzyl-, and 2-phenylethyl-arsonic acids, $R{\cdot}AsO_3H_2$; (ii) dimethyl-, diethyl-, and dibutyl-arsonous acids, $R_2AsO(OH)$; and (iii) 3-ethylamino-, 3-propylamino-, 3-butylamino-, 3-benzylamino-, 3-2′-phenylethylamino- and 3-*cyclo*hexylamino-propylarsonic acids, $R{\cdot}NH{\cdot}[CH_2]_3{\cdot}AsO_3H_2$.

Tissue cultures of mouse fibroblasts have been employed. The compounds were either applied to the cultures after active growth had commence or introduced into the media at the time of preparing the cultures, as neutral solutions in distilled water. Control cultures received the same amount of distilled water.

Most of the substances examined exhibited a mitotic poisoning over a relatively small range of concentrations. With each the aim has been to determine the concentration at which it interferes with the normal course of mitosis without appreciably damaging " resting cells."

The mitotic aberrations observed were of two main types, determined by whether the action was primarily on the mitotic spindle or on the chromosomes. The former results in the arrest of mitosis at metaphase, with the chromosomes either scattered throughout the cytoplasm or forming a cluster in the centre of the cell. This is the characteristic effect induced by colchicine. Of the substances listed below only dimethyl- and diethyl-arsonous acids and the thioarsinite formed from the former and cysteine have induced this mitotic aberration specifically. The action of the other arsenicals on the chromosomes is manifested by their distortion and fusion. Irregularities in the separation of the chromosomes to opposite poles of the mitotic spindle are extremely common. Chromosomes exhibit " stickiness," resulting in " bridges." Occasionally fragmentation is seen. Fusion of chromosomes may occur at metaphase or during anaphase. The most intense mitotic poisoning results in fusion of chromosomes at late prophase, thereby completely arresting mitosis.

The relative activities of the arsenicals examined were as given in the Table.

The following *N*-substituted derivatives of 3-aminopropylarsonic acid exhibited varying degrees of mitotic activity in dilutions between 1/10,000 and 1/25,000: *NN*-dimethyl, *N*-methyl, *N*-ethyl, *N*-butyl, *N*-hexyl, *N-cyclo*hexyl, *N*-benzyl, and *N*-2-phenylethyl. The last two were the best mitotic poisons in this group. One tervalent arsenical of this type was tested, namely, 3-(1-phenylethylamino)propylarsine dichloride, which was active at 1/100,000.

$Me_2AsO(OH)$	Cytological action similar to that of colchicine. Marked activity in dilutions of 1/50,000—1/75,000. Some activity at 1/100,000.
$Et_2AsO(OH)$	Not such a good mitotic poison as cacodylic acid. Active in concns. up to 1/100,000.
$Bu^n_2AsO(OH)$	Inactive within the range of dilutions used.
$Me_2As{\cdot}S{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$ ...	Better mitotic poison at 1/100,000 than cacodylic acid.

The remaining compounds act primarily on the chromosomes. The mitotic aberrations are not of the " colchicinic " type.

$Ph_2AsO(OH)$	Active over a wide range of concns. 1/50,000–1/200,000.		
$(p\text{-}NH_2{\cdot}C_6H_4)_2{\cdot}AsO(OH)$	Inactive within the range of dilutions employed.		
$Me{\cdot}AsO(OH)_2$	Comparable activity at		1/5,000
$Pr^n{\cdot}AsO(OH)_2$	,,	,,	1/75,000
$CH_2{:}CH{\cdot}CH_2{\cdot}AsO(OH)_2$	,,	,,	1/75,000
$Bu^n{\cdot}AsO(OH)_2$	,,	,,	1/700,000
$C_6H_5{\cdot}AsO(OH)_2$	,,	,,	1/10,000
$C_6H_5{\cdot}CH_2{\cdot}AsO(OH)_2$	,,	,,	1/50,000
$C_6H_5{\cdot}CH_2{\cdot}CH_2{\cdot}AsO(OH)_2$	,,	,,	1/100,000
$NH_2{\cdot}[CH_2]_3{\cdot}AsO(OH)_2$	,,	,,	1/10,000—1/25,000

Preparation of some of the arsenicals used in this investigation is described in the Experimental section. We are indebted to Professor F. Challenger of Leeds University for a gift of propylarsonic and diethylarsinic acids.

Experimental.

3-n-*Butylaminopropylarsonic Acid Hydrochloride.*—3-Chloropropylarsonic acid (6·07 g.), *n*-butylamine (17·6 c.c.), and alcohol (40 c.c.) were boiled under reflux for 12 hours. Alcohol and free butylamine were then removed by distillation, and complete removal of butylamine effected by addition of N-sodium hydroxide (10 c.c.) and distillation to dryness under reduced pressure. 3N-Hydrochloric acid (10 c.c.) was added and the solution again evaporated to dryness and finally extracted with absolute alcohol to remove sodium chloride. The alcoholic solution on concentration deposited glistening leaflets of 3-n-*butylaminopropylarsonic acid hydrochloride* (3·7 g.), which on recrystallization from boiling alcohol (20 c.c.) had m. p. 232° (Found: C, 30·8; H, 7·1; N, 4·8. $C_7H_{18}O_3NAs,HCl$ requires C, 30·5; H, 7·0; N, 5·1%).

3-n-*Propylaminopropylarsonic Acid Hydrochloride* (cf. Gough and King, *J.*, 1928, 2426).—*n*-Propylamine (11·0 g.) and 3-chloropropylarsonic acid (5·7 g.) were heated without extra solvent on the water-bath for 15 hours. The free propylamine was distilled off and the residue treated as above. 3-*n*-Propylaminopropylarsonic acid hydrochloride separated as microscopic plates which on crystallization from the minimum volume (5·5 parts) of boiling absolute alcohol gave the pure salt (2·66 g.), m. p. 210° in agreement with previously recorded data.

3-*Ethylaminopropylarsonic Acid Hydrochloride.*—3-Chloropropylarsonic acid (6·07 g.) and alcoholic ethylamine solution (24·3 g. of 33%) were heated in a sealed tube for 16 hours at 100°. Excess of ethylamine was removed by distillation under reduced pressure in the presence of strong aqueous sodium hydroxide, and the required *hydrochloride* was isolated from the neutralized solution by extraction with absolute alcohol and addition of acetone (yield, 3·0 g.). It crystallised from absolute alcohol in silky leaflets which, air-dried, melted indefinitely between 100° and 120° (Found: loss at 100°, 2·6. $C_5H_{14}O_3NAs,HCl,0{\cdot}5H_2O$ requires H_2O, 3·5%. Found, on anhydrous salt: C, 23·9; H, 6·2; Cl, 13·9. $C_5H_{14}O_3NAs,HCl$ requires C, 24·2; H, 6·1; Cl, 14·3%).

3-*Benzylaminopropylarsonic Acid Hydrochloride.*—3-Chloropropylarsonic acid (6·08 g.), benzylamine (19·5 c.c.), and absolute alcohol (20 c.c.) were boiled for 18 hours. When the reaction product was worked up as described above, *benzylaminopropylarsonic acid hydrochloride* (3·88 g.) separated from the alcoholic solution and on crystallization from the minimum volume (70 c.c.) of boiling absolute alcohol formed clusters of soft needles (3·45 g.), m. p. 240° (effervescence) (Found: C, 39·0; H, 5·8; N, 4·5. $C_{10}H_{16}O_3NAs,HCl$ requires C, 38·8; H, 5·5; N, 4·5%).

3-(2-*Phenylethylamino*)*propylarsonic Acid Hydrochloride.*—3-Chloropropylarsonic acid (5·66 g.) and 2-phenylethylamine (20·3 g.) were heated on a vigorously boiling water-bath for 15 hours. N-Sodium hydroxide (50 c.c.) was added and the solution extracted exhaustively with ether. The aqueous solution was then made just acid to Congo-red paper by N-hydrochloric acid and then evaporated to dryness. The residue was extracted with boiling absolute alcohol, and on concentration of the extract 3-(2-*phenylethylamino*)*propylarsonic acid hydrochloride* (7·0 g.) separated. It crystallized from the minimum volume (130 c.c.) of boiling absolute alcohol in leaflets, m. p. 247—248° with resolidification to the anhydride (Found: C, 40·8; H, 5·9; N, 4·5; As, 23·5. $C_{11}H_{18}O_3NAs,HCl$ requires C, 40·8; H, 5·9; N, 4·3; As, 23·2%).

3-cyclo*Hexylaminopropylarsonic Acid Hydrochloride.*—3-Chloropropylarsonic acid (6·07 g.) and *cyclo*hexylamine (20·7 c.c.) were heated on the vigorously boiling water-bath for 24 hours. Unchanged *cyclo*hexylamine having been removed by ether extraction from the solution made strongly alkaline, the aqueous solution was neutralized to Congo-red paper and evaporated to dryness. The required 3-cyclo*hexylaminopropylarsonic acid hydrochloride* (6·1 g.) was extracted with absolute alcohol. It required 3 volumes of absolute ethyl alcohol for recrystallization and separated in clusters of small pointed needles, m. p. 172—173° (Found: C, 36·3; H, 7·6; N, 4·7. $C_9H_{20}O_3NAs,HCl$ requires C, 35·8; H, 7·0; N, 4·6%).

2-*Amino*-2-*carboxyethyl Dimethylthioarsinite*.—Cysteine hydrochloride (4·73 g.) and cacodylic acid (1·38 g., 0·3 mol.) were each dissolved in water (10 c.c.) and the solutions mixed. Some heat was developed and after 5 minutes cystine began to separate. N-Sodium hydroxide solution (30 c.c.) was then added, with cooling. Finally the solution was saturated with carbon dioxide and kept for 12 hours. The cystine (2·4 g.) was collected. On concentration of the filtrate *in vacuo* over sulphuric acid, 2-*amino*-2-*carboxyethyl dimethylthioarsinite* (S-*dimethylarsinocysteine*) (4·25 g.) separated. It crystallized from water (8 c.c.) in shining needles, m. p. 219—220° (decomp.) (Found: C, 26·0; H, 5·6; N, 6·7; S, 13·6. $C_5H_{12}O_2NSAs$ requires C, 26·6; H, 5·4; N, 6·2; S, 14·2%). This thioarsinite slowly dissolves in sodium hydrogen carbonate solution and then gives no nitroprusside reaction, but if dissolved in sodium hydroxide solution it gives a strong reaction.

Benzylarsonic Acid.—This acid was prepared by the method of Quick and Adams (*J. Amer. Chem. Soc.*, 1922, **44**, 811) who give m. p. 167—168°, but in our hands it always had m. p. 196—197° (Found: C, 39·4; H, 4·0. Calc. for $C_7H_9O_3As$: C, 38·9; H, 4·2). Dehn and McGrath (*J. Amer. Chem. Soc.*, 1906, **28**, 354) also give m. p. 167°.

2-*Phenylethylarsonic Acid*.—Phenylethyl bromide (18·5 g.) and arsenious oxide (9·9 g.), dissolved in 10N-sodium hydroxide solution (30 c.c.), were boiled with vigorous stirring for 28 hours. The solution was extracted with ether, to remove unchanged phenylethyl bromide, and neutralized to litmus, and the arsenious oxide removed. When the filtrate was made acid to Congo-red paper, *phenylethylarsonic acid* (3·0 g.) separated. It crystallized from boiling water (50 c.c.) in thin hexagonal plates, m. p. 153° (Found: C, 42·2; H, 4·5. $C_8H_{11}O_3As$ requires C, 41·7; H, 4·8%).

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