haemopoietic stem cells has never been established and it has been suggested that they may even be indistinguishable from marrow lymphocytes. This contention lends support to the monophyletic theory of the origin of lymphoid and erythroid series[7,8] and if correct would also explain their antigenic similarity. An alternative explanation may be that thymocytes, as opposed to splenic lymph node or blood lymphocytes, were the source of antigen in the preparation of the ALS used in these experiments. Thymocytes are derived from the bone marrow[9], so the similarity in antigenic composition with haemopoietic stem cells may result from a common origin. It is possible that a greater degree of antigenic disparity may exist between marrow stem cells and true lymphoid cells which might be brought to light if the latter cells were used for immunization. Recent work has shown that thymocytes possess some antigens not present in other lymphoid cells[10].

These experiments give no explanation of the mechanism of inactivation by ALS of either of these two types of cell. Our (unpublished) observation, that the addition of guinea-pig complement does not enhance the inactivation of either cell type *in vitro* by dilutions of ALS down to 1 : 128, supports the view that the action of ALS is predominantly cytotoxic. If a process operated *in vivo* that did not depend on the action of the recipient's own complement, a synergistic effect could be expected between this process and the complement present during *in vitro* incubation. It is possible that coating the cell surface with antibody interferes with the homing property of haemopoietic stem cells to the bone marrow and of immunocompetent cells to the sites in lymphoid tissues in which they initiate a graft versus host reaction.

There is an unexplained discrepancy. On the one hand, ALS has an immediate effect on immunocompetent cells *in vitro*, as reported in these studies and by Brent *et al.*[11], who used mortality in mice injected neonatally with allogeneic lymph node cells as their assay system. On the other hand, a delay of 3–6 days has been reported to occur between the injection of ALS *in vivo* and maximal suppression of immune function, as demonstrated by the capacity of the animal to reject allogeneic skin grafts[12], and of lymph node cells to produce haemolysins and agglutinins to foreign red cells[13], and to induce fatal graft versus host disease[11]. Although injected ALS produces an immediate fall in number of circulating lymphocytes[14], it is apparently prevented from reaching extravascular lymphoid tissues for several days.

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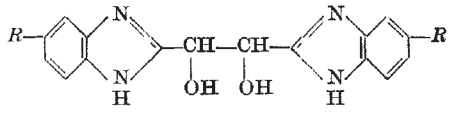
PATHOLOGY

Inhibitory Effect of 1,2-*bis*(2-Benzimidazolyl) 1,2-ethanediol Derivatives on Poliovirus

The inhibitory properties of 2-(α-hydroxybenzyl) benzimidazole (HBB) on the replication of poliovirus have been fully demonstrated[1,2]. O'Sullivan *et al.*[3] also reported that 1,2-*bis*(2-benzimidazolyl) 1,2-ethanediol (I) has a selective inhibitory action on the multiplication of poliovirus. On the basis of these findings, we have synthesized, 1,2-*bis*(2-benzimidazolyl) 1,2-ethanediol derivatives and tested them for their anti-poliovirus effect.



R = H (I); R = CH_3 (II); R = Cl (III); R = OCH_3 (IV)

1,2-*bis*(2-Benzimidazolyl) 1,2-ethanediol derivatives were prepared by the following procedures. *o*-Phenylenediamine or 4-methyl, 4-chloro, and 0·04 molar 4-methoxy *o*-phenylenediamine were dissolved in 50 ml. of 4 normal hydrochloric acid in a 300 ml. round flask. To this solution was added 0·02 molar tartaric acid and the mixture was refluxed gently on a sand-bath for 2–3 h. The reaction mixture was cooled externally with cold water and neutralized with 20–30 per cent sodium carbonate solution while stirring. The solution was kept in an ice-box overnight to complete crystallization. The crystals were collected by filtration and then recrystallized three times from dil. ethanol. The properties and yields of the compounds are given in Table 1.

The anti-poliovirus activity of these compounds was tested by using a poliovirus type 1 (Mahoney strain) and HeLa S_3 cells in the following way. A suspension of HeLa S_3 cells (4 ml. containing 2×10^5 cells/ml.) in YLE growth medium (1·0 g of yeast extract and 5·0 g of lact-albumin hydrolysate in 1,000 ml. of Earle's balanced salt solution, with 100 U of streptomycin and 500 U of penicillin supplemented with 10 per cent calf serum) was put into a plaque bottle, and incubated at 37° C for 4 days. After the monolayer cell sheet has been established, the growth medium was removed and, immediately after washing with phosphate buffered saline (PBS, *p*H 7·2), 0·05 ml. of different dilutions (10^{-3}–10^{-5} molar) of each tested compound and 0·45 ml. of the poliovirus dilution (30–40 PFU/bottle) were added. After incubation for 1 h at 37° C, the culture fluid was removed, and the cells were washed three times with PBS. Then 4·5 ml. of agar overlay medium and 0·5 ml. of the diluted compound were added in turn to the bottle. The agar overlay medium consisted of equal volumes of 1·8 per cent Bacto–Difco agar and twice concentrated YLE medium supplemented with 8 per cent calf serum and 0·0025 per cent (final concentration) neutral red. After incubation for 4 days at 37° C plaques were counted. For untreated control groups, the same amount of PBS was used instead of one of the compounds being tested. The results shown in Table 2 indicate that 1,2-*bis*(2-benzimidazolyl) 1,2-ethanediol derivatives clearly inhibit the replication of poliovirus. Compound IV showed complete inhibition.

The anti-poliovirus activity of compound IV was also examined by the $TCID_{50}$ estimating dilution method, as shown in Table 3, which indicates that the minimum inhibitory concentration of this compound was about 2×10^{-7} molar. This effect is about 1,000 times greater than that of HBB or guanidine[4]. The possibility that compound IV affected the cells themselves in any way can be excluded. Compound IV showed no toxicity in a

Table 1. PROPERTIES OF THE COMPOUNDS TESTED

Compound	Melting point* (° C)	Yield obtained (%)	Formula	Analysis Calculated	Analysis Found
(I) 1,2-*bis*(2-Benzimidazolyl) 1,2-ethanediol (ref. 3)	> 300	14		—	—
(II) 1,2-*bis*[5 (or 6)-Methyl-2-benzimidazolyl] 1,2-ethanediol	230–231	20	$C_{18}H_{18}N_4O_2.H_2O$	C = 63·51 H = 5·92 N = 16·46	C = 63·53 H = 6·06 N = 16·30
(III) 1,2-*bis*[5 (or 6)-Chloro-2-benzimidazolyl] 1,2-ethanediol	219–220	13	$C_{16}H_{12}N_4O_2Cl_2.H_2O$	C = 50·41 N = 14·70	C = 50·65 N = 14·68
(IV) 1,2-*bis*[5 (or 6)-Methoxy-2-benzimidazolyl] 1,2-ethanediol	213–214	23	$C_{18}H_{18}N_4O_4.H_2O$	H = 5·41 N = 15·05	H = 5·64 N = 15·01

* Decomposition point is shown.

Table 2. EFFECT OF 1,2-*bis*(2-BENZIMIDAZOLYL) 1,2-ETHANEDIOL DERIVATIVES ON REPLICATION OF POLIOVIRUS

Compounds No.	Added* amounts (molar)	No. of plaques (mean and dispersion) Untreated groups	Treated groups	Percentage inhibition† (mean and dispersion)
I	10^{-3}	37·0 ± 1·87 (35, 35, 38, 38, 39)	10·2 ± 1·30 (9, 9, 10, 11, 12)	72·6 (76·9–65·6)
	10^{-4}	33·6 ± 1·14 (32, 34, 34, 33, 35)	11·0 ± 3·35 (7, 8, 11, 14, 15)	67·2 (80·0–53·1)
	10^{-5}	34·4 ± 2·62 (32, 33, 34, 35, 38)	21·4 ± 3·19 (18, 18, 22, 24, 25)	37·8 (52·6–21·8)
	10^{-6}	36·0 ± 2·45 (34, 34, 37, 39)	31·5 ± 2·64 (28, 31, 33, 34)	12·5 (28·2–0·0)
II	10^{-3}	35·0 ± 1·00 (34, 34, 35, 36, 36)	6·8 ± 2·77 (3, 5, 8, 8, 10)	80·6 (91·6–70·6)
	10^{-4}	30·0 ± 2·55 (28, 28, 29, 31, 34)	7·2 ± 2·58 (4, 7, 6, 8, 11)	76·0 (88·2–71·4)
	10^{-5}	31·2 ± 1·90 (28, 31, 32, 32, 33)	21·0 ± 0·70 (20, 21, 21, 21, 22)	32·7 (39·4–21·4)
	10^{-6}	27·0 ± 3·29 (24, 24, 27, 29, 31)	24·5 ± 2·44 (22, 23, 26, 27)	9·8 (24·1–0·0)
III	10^{-4}	45·6 ± 3·03 (42, 43, 46, 48, 49)	2·4 ± 1·51 (0, 2, 3, 3, 4)	92·4 (100·0–90·4)
	10^{-5}	48·0 ± 0·70 (47, 48, 48, 48, 49)	32·8 ± 3·14 (30, 31, 32, 33, 38)	31·7 (38·7–29·8)
	10^{-6}	48·0 ± 2·55 (45, 46, 48, 50, 51)	36·6 ± 2·70 (33, 35, 37, 38, 40)	23·8 (35·3–11·1)
IV	10^{-4}	31·8 ± 0·83 (31, 31, 32, 32, 33)	0·0 ± 0·00 (0, 0, 0, 0, 0)	100·0 (100–100)
	10^{-5}	34·6 ± 1·52 (33, 34, 34, 35, 37)	0·0 ± 0·00 (0, 0, 0, 0, 0)	100·0 (100–100)
	10^{-6}	36·0 ± 2·12 (34, 34, 36, 37, 39)	0·0 ± 0·00 (0, 0, 0, 0, 0)	100·0 (100–100)

* Final molar concentration is shown.

† $\frac{\text{No. of plaques of the untreated group} - \text{No. of plaques of the treated group}}{\text{No. of plaques of the untreated group}} \times 100$

concentration of 10^{-4} molar in 7 days, but in a concentration of 10^{-3} molar cells were degenerated or separated from tissue culture tubes. Recent results (reported at the Fifteenth General Meeting of the Society of Japanese Virologists in October 1967 at Chiba) show that (*a*) the incorporation of 2-^{14}C-uridine and ^{3}H-valine did not differ between cell cultures without compound IV and cultures treated with 10^{-4} molar compound IV, and (*b*) compound IV strongly reduced the yield of intracellular virus. This compound can therefore be supposed to be non-toxic.

Table 3. ANTI-POLIOVIRUS ACTIVITY OF 1,2-BIS[5(OR 6)-METHOXY-2-BENZIMIDAZOLYL] 1,2-ETHANEDIOL

Added* amount	$-\log TCID_{50}$ Untreated groups	Treated groups	Difference $-\log TCID_{50}$
10^{-4}	9·00	0·75	8·25
10^{-5}	9·00	1·25	7·75
10^{-6}	9·00	1·50	7·50
10^{-7}	9·00	8·75	0·25
10^{-8}	9·00	9·00	0·00

* Final molar concentration is shown.

The high inhibitory effect of this compound should be valuable not only for anti-viral chemotherapy but for analysing the mechanism of viral replication.

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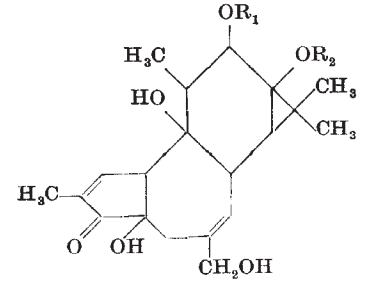
Response of an Active Component of Croton Oil to Short-term Tests of Carcinogenicity

THE cocarcinogenic properties of croton oil have been known for a long time[1], and there have been many attempts to identify its active components[2,3].

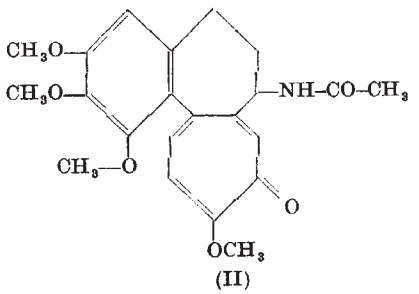
Hecker *et al.*[3–8] succeeded in isolating a set of phorbol esters from croton oil. According to these authors phorbol is a tetracyclic diterpene: $C_{20}H_{28}O_6$. Its structural formula is now known to correspond to the formula (I) shown[8], where $R_1 = R_2 = H$. Hecker's experiments showed that these esters, apart from being promoters of cancer, also have a mild but definite tumour initiating effect[9].

Among the isolated esters of phorbol, Hecker's substance A_1 (ref. 5) proved to be the most powerful. This product has the same melting point (72° C) as "substance *C*-3" of Arroyo and Holcomb[10] and the same mass spectrum as "substance *C*" of Van Duuren and Orris[2]. Substance A_1 is supposed to be *c*-acetyl-*b*-myristoyl-phorbol (that is, 13-O-acetyl-phorbol-12-myristate): $C_{36}H_{56}O_8$, corresponding to formula (I), where R_1 is a myristoyle, and R_2 an acetyl residue[5,8].

We have investigated the response of Hecker's substance A_1 to the short-term tests of carcinogenicity. Two tests—the sebaceous gland suppression test and the epidermal hyperplasia test—were made by our usual



(I)



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