Note

Antitumor Activity of 5-Hydroxymethyl-2-furoic Acid[†]

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Received April 6, 1981

In our screening studies for the selective inhibitors against SV40-transformed cells, 5-hydroxymethyl-2-furoic acid was isolated from the ethylacetate extract of the cultured broth and mycelium of *Pyricularia grisea* IAM 5051. The isolated 5-hydroxymethyl-2-furoic acid (65-I) showed a selective cytotoxicity against SV40-transformed cells (*in vitro*) and an antitumor activity against Sarcoma 180 (*in vivo*).

The 65-I substance was isolated from jar fermentation carried out in the following procedures: One hundred ml of the medium in a 500 ml rotary shaking flask, containing of 3.0% glucose, 1.0% malt extract, 0.5% peptone, 0.2% yeast extract, 0.1% KH_2PO_4 , 0.1% NH_4Cl , and 0.04% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, was inoculated with *Pyricularia grisea* grown on an agar slant and rotary-shaking cultured at 28°C for 96 hr. One hundred ml of this culture was then transferred into 30 liter jar fermentor containing 15 liter of the same components of seed medium, and cultured at 28°C for 96 hr under an equal volume of aeration with agitation at 250 rpm.

The filtrate of cultured broth and mycelium collected by centrifugation was extracted with ethylacetate at room temperature. Ethylacetate extract was dried over anhydrous sodium sulfate and concentrated *in vacuo* to remove the ethylacetate. Purification was carried out by silica gel column (Merck) chromatography. The selective cytotoxic fraction was detected by N/T assay.¹⁾

The 65-I substance was colorless plate crystal, and mp 115°C. It was readily soluble in pyridine and dimethyl sulfoxide, and slightly soluble in methanol. The IR spec-

trum in a KBr pellet indicated the presence of carbonyl at 1660, alcohol at 3280 and 1300, and ether at 1220 cm^{-1} . The molecular formula, $\text{C}_6\text{H}_6\text{O}_4$, was assigned for the 65-I substance on the basis of elementary analysis and the molecular weight, 142, determined by mass spectroscopy. MS m/z (intensity %): 143 ($\text{M}^+ + 1$, 5), 142 (M^+ , 92.4), 123 (40.3), 113 (32.8), 95 (14.3), 79 (18.5), 69 (61.3), 51 (16.8), 41 (100). $^1\text{H-NMR}$ $\delta_{\text{TMS}}^{\text{Pyridine-d}_5}$ ppm: 8.61 (2H, s), 7.14 (1H, d), 6.41 (1H, d), 4.70 (2H, s). $^{13}\text{C-NMR}$ $\delta_{\text{TMS}}^{\text{Pyridine-d}_5}$ ppm: 161.21 (1C, s*), 159.67 (1C, s*), 145.69 (1C, s*), 118.22 (1C, d*), 108.22 (1C, d*), 56.89 (1C, s*). (*; Multiplication in ^1H -off resonance spectrum: s, singlet; d, doublet.) All this evidence indicated that the 65-I substance is 5-hydroxymethyl-2-furoic acid.

Selective cytotoxic activity against SV40-transformed cells *in vitro* was assayed by N/T assay.¹⁾ As shown in Table I, 5-hydroxymethyl-2-furoic acid had a selective cytotoxicity from the first day, but the minimum cytotoxic concentration against SV40-transformed cells increased with the treatment time.

Antitumor activity *in vivo* was assayed using the total packed cell volume (T.P.C.V.) method.²⁾ The ascitic tumor cells of Sarcoma 180 (10^7 cells) were implanted intraperitoneally in ICR mice, 5 week old, weighing 17.5~20.0 g. Saline solution of the samples was administered once daily for 5 successive day (50 mg/kg $\times$ 5). On the seventh day, the mice were sacrificed, all the ascites collected, and the volume of tumor cells in the ascites measured. Thus, we obtained the ratio of the T.P.C.V. for the test group to that for the control group inoculated with saline. This ratio was called T/C. The T/C % of the 65-I substance was 36.6, and the judgement of antitumor activity was ++ (slightly effective).

Acknowledgment. We wish to thank Mr. N. Yokomizo, Research and Development Laboratories, Sapporo Breweries Ltd., for the assay of the antitumor activity *in vivo*.

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[†] The Selective Inhibitors against SV40-transformed Cells. Part II.

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TABLE I. CYTOTOXICITY OF 5-HYDROXYMETHYL-2-FUROIC ACID AND 2-FUROIC ACID

Compound	5-Hydroxymethyl 2-furoic acid (65-I)						2-Furoic acid*					
	C3H-2K			SV40-C3H-2K			C3H-2K			SV40-C3H-2K		
Cell												
Days of treatment	1	2	3	1	2	3	1	2	3	1	2	3
2000 mcg/ml	—	—	—	+	++	+++	—	—	—	+	++	++
1000	—	—	—	+	++	++	—	—	—	+	++	++
500	—	—	—	+	++	++	—	—	—	+	+	+
250	—	—	—	+	+	±	—	—	—	+	±	—
125	—	—	—	±	±	±	—	—	—	—	—	—
62.5	—	—	—	—	—	—	—	—	—	—	—	—
31.3	—	—	—	—	—	—	—	—	—	—	—	—

Cytotoxicity is expressed as follows; —, no toxic effect; ±, slight toxic effect; +, cells expanded, a small number of detached cells; ++, mixture of expanded and rounded cells; +++, a large number of rounded and detached cells.

* Commercial compound (Tokyo Kasei Co.).

TABLE II. ANTITUMOR ACTIVITY OF 5-HYDROXYMETHYL-2-FUROIC ACID AND 2-FUROIC ACID

Compounds	Dose (mg/kg/day)	Body wt. gain (g)	Vol. of Ascites	% of tumor cells	TPCV	T/C*	Activity**
5-Hydroxymethyl 2-furoic acid	50	+1.6	3.7	17.5	0.63	36.6	++
2-Furoic acid***	50	+3.2	2.4	32.5	0.84	48.8	+
Control	0	+5.7	5.7	31.0	1.72		

* Ratio of total packed cell volume for test group to that for control group given saline.

** +++, T/C < 10; ++, T/C 11–40; +, T/C 41–65; —, T/C > 65.

*** Commercial compound (Tokyo Kasei Co.).