Potential Anticancer Agents. III. 2-Phthalimidoaldehydes and Derivatives[1a]

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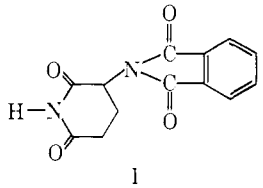
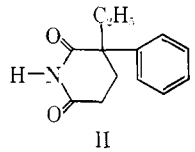
Aliphatic aldehydes containing a phthalimido group in position 2 of the chain were synthesized by catalytic hydrogenation of the corresponding 2-phthalimidoacyl chlorides. Various carbonyl derivatives of these 2-phthalimidoaldehydes were also prepared. Several of the phthalimidoaldehydes and particularly the thiosemicarbazone and isonicotinylhydrazone derivatives exhibited sufficient inhibitory activity *vs.* the Ehrlich ascites carcinoma in mice to warrant further study.

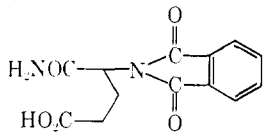
Aldehydes, such as heptanal,[2] propanal,[3] glyceraldehyde,[3] and citral,[2d,4] and 2-ketoaldehydes, such as 3-ethoxy-2-ketobutanal,[5] have been shown to possess some antitumor activity. The phthalimido moiety might also aid in inhibiting tumors since it seems to have an influence on cell growth. N-(2,6-Dioxo-3-piperidyl)phthalimide[6] (I), which contains the phthalimido group, has caused many cases of fetal malformations[7] and has been shown to inhibit leucocyte multiplication,[8] whereas 2-ethyl-2-phenylglutarimide[9] (II), which has a structure similar to N-(2,6-dioxo-3-piperidyl)phthalimide but lacks the phthalimido moiety, does not have these effects.[10]



I



II



III

Since 4-phthalimidoglutaramic acid (III) is the main metabolic product of N-(2,6-dioxo-3-piperidyl)phthalimide excreted by man,[10] the intact phthalimido group may exert biological activity. Although tests of N-(2,6-dioxo-3-piperidyl)phthalimide against tumors showed that it has weak or no antitumor activity,[11] it seemed worthwhile to prepare and test other compounds containing the phthalimido moiety. In this paper, syntheses and antitumor tests are described for the following 2-phthalimidoaldehydes: 2-phthalimidobutanal, 2-methyl-2-phthalimidopropanal, 2-phthalimidopentanal, and 3-methyl-2-phthalimidopentanal.

Several derivatives of aldehydes have also been shown to inhibit the growth of neoplasms, for example, pyridine-2-carboxaldehyde thiosemicarbazone,[12] 3-ethoxy-2-ketobutanal bisthiosemicarbazone,[13] octadecylthiosemicarbazones of aldehydes,[14] 4-hydroxy-2-thiopyrimidine-6-carboxaldehyde thiosemicarbazone,[15] indole-3-carboxaldehyde *p*-bromophenylhydrazone,[16] and 4-hydroxy-2-thiopyrimidine-6-carboxaldehyde benzenesulfonylhydrazone and tosylhydrazone.[15] Therefore, the following derivatives of the 2-phthalimidoaldehydes were also prepared for biological studies: thiosemicarbazone, phenylsemicarbazone, 2,4-dinitrophenylsemicarbazone, phenylhydrazone, 2,4-dinitrophenylhydrazone, benzenesulfonylhydrazone, tosylhydrazone, isonicotinylhydrazone, 2-amino-4-hydrazono-6-methylpyrimidine, and 2-ethylthio-4-hydrazono-6-methylpyrimidine.

Experimental Section[17]

2-Phthalimido Acids.—The 2-phthalimido acids prepared as intermediates for the syntheses of the 2-phthalimidoacyl chlorides and 2-phthalimidoaldehydes were the following: 2-phthalimidobutanoic, 2-phthalimidopropionic, 2-phthalimidopentanoic, and 3-methyl-2-phthalimidopentanoic acids. These were prepared according to the method of Ulrich[18] by fusing phthalic anhydride and the appropriate α-amino acid in the absence of solvent. These 2-phthalimido acids have been previously reported by other workers.[19]

2-Phthalimidoacyl Chlorides.—The 2-phthalimidoacyl chlorides were prepared by treating the appropriate 2-phthalimido acid with excess $SOCl_2$ (2.5 moles to 1 mole of acid) in the absence of solvent at room temperature until the acid had completely dissolved. The excess $SOCl_2$ was then removed by distillation under high vacuum. If the phthalimidoacyl chloride was a liquid, it

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(17) Elemental analyses, melting points and refractive indices of the synthesized compounds are given in Table I. The elemental analyses were performed by Alfred Bernhardt Mikroanalytisches Laboratorium, Mülheim (Rühr), West Germany. The melting points were taken with a Fisher-Johns melting point apparatus and have been corrected. The refractive indices were taken with a Bausch and Lomb refractometer.

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TABLE I

ELEMENTAL ANALYSES, MELTING POINTS, AND REFRACTIVE INDICES

No.	Compd	Mp, °C or $n^{25}D$	% C		% H		% N		% S	
			Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
1	2-Phthalimidobutanol	111.0–112.5	66.34	66.36	5.10	5.08	6.46	6.61		
2	2-Methyl-2-phthalimidopropanal	90.1–90.6	66.34	66.33	5.10	5.19	6.46	6.51		
3	2-Phthalimidopentanal	1.5555	67.51	67.46	5.67	5.83	6.06	6.07		
4	3-Methyl-2-phthalimidopentanal	1.5608	68.53	68.42	6.17	6.31	5.72	5.71		
5	2-Phthalimidobutanal thiosemicarbazone	209.5–209.7	53.76	53.69	4.86	5.30	19.22	19.16	11.04	10.96
6	2-Methyl-2-phthalimidopropanal semicarbazone	218.2–219.4	53.76	53.65	4.86	4.99	19.22	19.39	11.04	10.95
7	2-Phthalimidopentanal thiosemicarbazone	210.4–211.5	55.24	55.17	5.29	5.70	18.40	18.31	10.53	10.32
8	3-Methyl-2-phthalimidopentanal thiosemicarbazone	207.0–209.4	56.64	56.59	5.69	6.13	17.55	17.48	10.07	9.96
9	2-Phthalimidobutanal phenylsemicarbazone	157.2–157.9	65.13	65.21	5.19	5.44	16.00	15.98		
10	2-Phthalimidobutanal 2,4-dinitrophenylsemicarbazone	242.0–243.2	51.82	51.50	3.66	3.89	19.09	18.92		
11	2-Phthalimidopentanal 2,4-dinitrophenylsemicarbazone	238.0–238.6	52.86	52.52	3.99	4.05	18.50	18.61		
12	3-Methyl-2-phthalimidopentanal 2,4-dinitrophenylsemicarbazone	220.6–221.2	53.84	53.62	4.30	4.46	17.94	17.28		
13	2-Methyl-2-phthalimidopropanal phenylhydrazone	123.0–123.6	70.34	69.86	5.57	5.55	13.67	14.11		
14	2-Phthalimidobutanal 2,4-dinitrophenylhydrazone	181.1–181.5	54.40	54.59	3.80	3.87	17.62	17.51		
15	2-Methyl-2-phthalimidopropanal 2,4-dinitrophenylhydrazone	256.3–256.7	54.40	53.31	3.80	3.84	17.62	17.83		
16	2-Phthalimidopentanal 2,4-dinitrophenylhydrazone	153.8–154.5	55.47	55.30	4.17	4.21	17.03	17.20		
17	2-Phthalimidobutanal benzenesulfonylhydrazone	139.1–140.4	58.20	58.18	4.61	4.71	11.31	11.45	8.63	8.43
18	2-Methyl-2-phthalimidopropanal benzenesulfonylhydrazone	153.3–154.6	58.20	58.22	4.61	4.91	11.31	11.38	8.63	8.54
19	2-Phthalimidobutanal tosylhydrazone	161.2–162.1	59.20	59.15	4.98	4.88	10.90	10.86	8.31	8.27
20	2-Phthalimidobutanal isonicotinylhydrazone	143.8–145.4	64.27	64.31	4.47	4.87	16.60	16.64		
21	2-Phthalimidopentanal isonicotinylhydrazone	162.1–163.2	65.13	65.31	5.18	4.98	15.99	15.81		
22	2-Amino-6-methyl-4-(2′-phthalimidopentanal hydrazono)pyrimidine	206.9–207.7	61.35	61.10	5.72	6.16	23.85	23.96		
23	2-Amino-6-methyl-4-(3′-methyl-2′-phthalimidopentanal hydrazono)-pyrimidine	114.9–116.3	62.27	62.08	6.00	6.31	22.90	22.76		
24	2-Ethylthio-6-methyl-4-(2′-phthalimidobutanal hydrazono)pyrimidine	67.3–68.1	59.51	59.22	5.52	5.86	18.27	18.16	8.36	8.46

was used in the hydrogenation without further purification, but if a solid, it was purified by crystallization from a mixture of ether and petroleum ether (bp 30–60°).

2-Phthalimidoaldehydes.—All the 2-phthalimidoaldehydes were synthesized by the Rosenmund reduction[20] of the 2-phthalimidoacyl chlorides, using 10% Pd–C catalyst. The procedure was similar to that described by Foye and Lange.[21] 2-Methyl-2-phthalimidopropanal was obtained in a 55.7% yield, whereas the other aldehydes were obtained in 92–95% yields.

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Carbonyl Derivatives of the 2-Phthalimidoaldehydes.—The thiosemicarbazone, phenylsemicarbazone, 2,4-dinitrophenylsemicarbazone, phenylhydrazone, 2,4-dinitrophenylhydrazone, benzenesulfonylhydrazone, tosylhydrazone, 2-amino-4-hydrazono-6-methylpyrimidine, and 2-ethylthio-4-hydrazono-6-methylpyrimidine derivatives of the 2-phthalimidoaldehydes (Table I) were prepared by heating the aldehyde and the appropriate carbonyl reagent in alcohol according to classical preparative procedures which have been described in detail by Craig.[22] The isonicotinylhydrazones were prepared in the absence of solvent.

Biological Testing.—The 2-phthalimidoaldehydes and their derivatives were tested *vs.* the Ehrlich ascites carcinoma in Swiss Webster white mice by a slight modification of procedures described previously.[23] Each mouse (initial weight approximately 30 g) received an intraperitoneal injection of 0.1 ml of pooled ascitic fluid (collected from donor mice which had borne the ascites carcinoma for 7–9 days) which had been diluted with 0.9% NaCl to a cell concentration of 10% by volume, based upon the results of an initial ascitocrit determination. The 0.1-ml inoculum contained an average of 7×10^6 carcinoma cells. For each assay, the mice were divided into a control group of eight mice and several (usually four or five) experimental groups of eight mice each. Twenty-four hours after the inoculation, each control mouse received an intraperitoneal injection of 0.2 ml of propylene glycol and each mouse in the experimental groups received an intraperitoneal injection of 0.2 ml of a propylene glycol solution of the compound to be tested. The intraperitoneal injections of control and experimental mice were continued twice daily for 6 days (total of 11 injections). On the 7th day all surviving mice in control and experimental groups were sacrificed. The volume of ascitic fluid was measured for each animal and percentage of cells by volume (ascitocrit) was determined for each sample of ascitic fluid by centrifugation in heparinized capillary tubes. The total packed-cell volume (TPCV) of tumor cells was calculated in each case, together with average values and standard deviations. Results for the more active compounds are recorded in Table II. Dosages are expressed as mg injected/kg/day and the mice were weighed daily. For control mice the average gain in carcass weight (after removal of ascitic fluid) was 1.2 g for the total assay period and consequently the total weight changes recorded in Table II can be attributed principally to the weight of ascitic fluid and tumor cells which accumulated in the peritoneal cavities of the mice during the assay period. However, the determination of TPCV of tumor cells is considered to be a more accurate method for assaying the effectiveness of the compounds.

TABLE II

RESULTS OF SCREENING TESTS *vs.* EHRLICH ASCITES CARCINOMA[a]

No.	Dose, mg/kg/day	Av body wt change T/C, g	Mortality during assay C	Mortality during assay T	Av T/C, ml	TPCV Std dev of T ± ml
2	66.2	−0.1/+3.8	0/8	2/8	0.99/2.53	0.46
2	39.2	+5.3/+6.4	0/8	3/8	1.54/2.19	0.17
5	81.0	−2.4/+4.4	3/8	4/8	1.98/3.67	0.28
5	40.0	+0.6/+5.1	1/8	1/8	1.3/2.2	0.15
6	68.0	+0.4/+5.8	3/8	3/8	1.09/4.62	0.51
6	32.5	+0.6/+4.8	1/8	1/8	1.3/3.1	0.36
7	66.4	−1.9/+5.8	3/8	1/8	0.74/4.62	0.44
7	31.0	−0.2/+4.6	1/8	1/8	0.78/2.6	0.21
8	68.0	−1.7/+5.8	3/8	3/8	0.37/4.62	0.14
8	32.0	+0.5/+5.6	1/8	2/8	0.87/2.9	0.32
8	15.2	+3.2/+2.8	0/8	0/8	0.71/1.47	0.53
14	64.8	−1.0/+7.7	0/8	4/8	0.58/2.26	0.03
14	25.9	+4.9/+6.0	1/8	2/8	0.73/2.08	0.52
15	67.6	+1.9/+6.8	0/8	4/8	1.57/2.93	0.43
15	58.6	−1.2/+2.8	0/8	2/8	0.46/1.47	0.56
15	30.0	−0.2/+4.1	1/8	1/8	1.33/2.42	0.24
21	68.7	−3.4/+6.4	1/8	3/8	0.36/1.35	0.14
21	32.7	+1.2/+5.9	1/8	2/8	1.54/2.87	0.30
21	11.3	+2.8/+6.4	0/8	1/8	1.29/2.19	0.36

[a] T = treated group; C = controls; TPCV = average total packed-cell volume of tumor cells on final day of assay. The standard deviation of TPCV for all controls was ±0.35 ml.

Summary of Results

Although a majority of the tested phthalimidoaldehydes and their derivatives showed inhibition of the Ehrlich ascites carcinoma, some of these compounds were effective only at relatively high dosages which gave evidence of general toxicity to the mice in terms of a higher mortality than that exhibited by the corresponding control group. Only the most effective compounds are listed in Table II. In general, the thiosemicarbazones, 2,4-dinitrophenylhydrazones, and isonicotinylhydrazones were more effective inhibitors of the tumor than the corresponding free aldehydes, although the aldehydes did exhibit activity and **2** was moderately effective. The 2,4-dinitrophenylhydrazones and isonicotinylhydrazones tended to be more toxic to the host animal in relation to corresponding controls than did the thiosemicarbazones which, in general, were the most effective compounds of the series. In the latter group, **8** was the most active compound. From the standpoint of inhibition of the tumor cells, **21** also was quite effective although slightly more toxic to the host mice than **8**. The latter compound was also tested in another type of assay in order to determine whether it would prolong the survival time of mice inoculated with the Ehrlich ascites carcinoma. Tumor-bearing mice (inoculated as described for the other type of assay) injected intraperitoneally with this compound in propylene glycol at a dosage of 15 mg/kg/day for 7 days had an average survival time of 17 ± 4 days in comparison with 10 ± 3 days for tumor-bearing control mice injected with propylene glycol alone. This compound at an intraperitoneal dosage of 20 mg/kg/day for 10 days also inhibited by approximately 50% the growth of the Ehrlich carcinoma when the tumor was transplanted as a solid tumor by subcutaneous injection into the axillary region of control and treated mice. It was of interest to compare the effectiveness of **8** in the total packed-cell volume assay with a compound of recognized activity *vs.* the Ehrlich ascites carcinoma. At a dose of 14.5 mg/kg/day for 6 days 5-fluorouracil gave a value of 0.17/1.50 ml for T/C in the TPCV assay in comparison with 0.61/1.50 for the thiosemicarbazone at a dose of 15 mg/kg/day. Thus, the thiosemicarbazone is less effective than 5-fluorouracil at comparable dosage, but it shows sufficient activity to warrant further study of this and related compounds, particularly with respect to mechanism of action.

Acknowledgment.—We wish to thank Mr. Ernest M. Walker for assistance in the screening tests.

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