difference was found in organ weights between control and treated rats. The following organs were removed and weighed: adrenals, liver, thyroid, pituitary, uterus, ovaries, testes, pancreas, spleen, and thymus.

Thus, the decrease in serum-cholesterol levels reported by Wachtel is unexplainable; however, various other fractions isolated from pituitary have been reported to cause an increase in serum-cholesterol levels in the dog,[1a] and these findings are consistent with the presence of cholesterol in the pituitary gland.

Only a limited study of the physical and chemical properties of II and III could be undertaken and this is described in the Experimental section. While it has not been possible, with the quantities of materials available to us, to fully identify either of these fractions, the data of fraction II suggests that it may be an N-acyl-sphingosine derivative.

Experimental

General.—Untrimmed bovine pituitary glands (912 g.) were collected over a 2-week period[2] and frozen until used. The glands were trimmed and dissected[3] to yield 71 g. of posterior lobe and 435 g. of anterior lobe.

Work-up of Posterior Lobe.—The posterior lobes (71 g.) were ground in a Waring blendor with acetone (500 ml.) and kept at room temperature in the dark for 1 week with occasional shaking. The acetone extracts were separated from proteinaceous material by filtration, and evaporated to dryness *in vacuo* at 50°. Traces of water were removed by successive additions and evaporations of absolute ethanol. The total residue weighed 2.75 g. and was soluble in freshly distilled methanol-free chloroform (*cf.* ref. 1b). The residue was put on a column of 150 g. of Woelm Activity II, neutral alumina with hexane. Two fractions were obtained on elution. The first was eluted with 1:1 benzene-ether. It was crystallized three times from petroleum ether yielding 430 mg., m.p. 148–150°, and was identified as cholesterol by rotation, mixture melting points, and infrared spectrum. The second fraction moved down the column as a sharply defined band, visible on irradiation with ultraviolet light but not to the naked eye. It was eluted with 1:20 methanol–chloroform yielding 120 mg. and was crystallized from methanol four times to yield 11 mg., m.p. 84–86° (fraction II). Further elution with pure methanol removed no other material from the column.

Work-up of Anterior Lobe.—The anterior lobes (435 g.) were extracted with acetone as described above for posterior lobes to yield 16 g. of an oily residue. Trituration with hexane yielded a hexane-insoluble portion (1.0 g.) which was shown to be identical with fraction II (m.p. 84–86°) obtained from work-up of the posterior lobes. The hexane-soluble portion was chromatographed on 600 g. of Woelm Activity II, neutral alumina to yield 3 fractions. The first eluted with 1:1 benzene–ether was shown to be cholesterol (1.9 g.) by melting point, mixture melting point, and infrared spectrum. The second, eluted with 1:20 methanol–chloroform, was shown to be identical with fraction II, obtained from posterior lobes and from the hexane-insoluble portion of the acetone extract of anterior lobes. It weighed 850 mg., m.p. 84–87°. The third fraction was eluted with 1:10 methanol–chloroform and weighed 465 mg. (III). No further material was obtained from the column.

Fraction II.—Fraction II, m.p. 84–86°, has λ_{max}^{hexane} 190 mμ (ϵ 160, c 1%) when run in a nitrogen atmosphere. The infrared spectrum was run in KBr and had a broad band centered at 3300 cm.$^{-1}$ (OH, NH) and bands at 1645 (amide), 1620 (C=C), and 720 cm.$^{-1}$ (paraffin chain). The n.m.r. spectrum run in deuteriochloroform at 60 Mc./sec. with tetramethylsilane as standard shows a high proportion (22:1) of methylene groups (τ 8.72) to methyl groups (τ 9.12). Fraction II moved as a single spot on thin layer chromatography on silica gel with 1:5 2-propanol–carbon tetrachloride.

Anal. Found: C, 76.21; H, 11.61; N, 2.87; O, 9.41.

Fraction III.—Fraction III (465 mg.) was rechromatographed to yield 248 mg. of material. It was an oil which crystallized

(2) Courtesy of Canada Packers Limited, Montreal.
(3) Courtesy of Dr. E. Greselin and Staff, Pathology Department.

from ether or from ethyl acetate at low temperatures. The crystals were waxy in appearance and melted well below room temperature. The material does not sublime. The infrared spectrum shows a strong carbonyl band at 1755 cm.$^{-1}$ and the ultraviolet spectrum shows two broad bands at λ_{max}^{EtOH} 275 mμ (ϵ 10.1, c 1%) and 225 (31.9, c 1%).

Long-Chain Thiosemicarbazones as Potential Anticancer and Antiviral Agents

BENJAMIN PRESCOTT

National Institute of Allergy and Infectious Diseases

AND CHEN PEIN LI

Division of Biologics Standards, National Institute of Health, Bethesda 14, Maryland

Since the initial report by Domagk[1] that certain aromatic aldehyde thiosemicarbazones possessed high antituberculous activity, numerous aliphatic, aromatic, and heterocyclic thiosemicarbazones have been synthesized[2,3] and tested as potential antituberculous agents. Many thiosemicarbazones have also been tested for antiviral activity. Hamre and co-workers[4,5] have reported that *p*-aminobenzaldehyde-3-thiosemicarbazone possessed antiviral activity, causing a significant delay in death and survival of a small percentage of chick embryos and mice infected with vaccinia virus. Bauer and Sheffield[6] showed that isatin-β-thiosemicarbazone was capable of protecting mice against infections with lethal doses of the IHD strain of neurotropic vaccinia virus. This report is concerned with the synthesis and characterization of 34 thiosemicarbazones, in particular various derivatives of long-chain thiosemicarbazones as potential anticancer and antiviral agents. Studies with the series of 4-octadecyl-3-thiosemicarbazones reported herein have shown that certain derivatives have some effect as potential anticancer and antiviral agents. Furthermore, because of low toxicity, they may be administered in larger does than are commonly used in the administration of various anticancer drugs.

Experimental

Chemical.—The octadecyl isothiocyanate was synthesized from octadecylamine according to the method of Moore and Crossley.[7] This intermediate was condensed with hydrazine by a standard method according to Pulvermacher[8] to give octadecylthiosemicarbazide. All the aldehydes employed were commercial preparations. The 4-octadecyl-3-thiosemicarbazones were prepared easily by the following procedure.

A solution of the aldehyde (0.1 mole) in ethanol (50 ml.) was added to a warm solution of the octadecylthiosemicarbazide

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TABLE I
OCTADECYLTHIOSEMICARBAZONES: CHEMICAL AND PHYSICAL PROPERTIES

Aldehyde	M.p., °C[a]	Empirical formula	Calcd. C	H	N	Found C	H	N
Valeraldehyde	44	$C_{24}H_{49}N_3S$	70.00	12.00	10.21	70.44	12.37	10.55
2-Ethylhexanal	35	$C_{27}H_{55}N_3S$	71.46	12.21	9.26	71.65	12.32	9.09
Glutaraldehyde	52	$C_{43}H_{86}N_6S_2$	68.74	11.54	11.18	68.87	10.83	10.89
4-Fluorobenzaldehyde	80	$C_{26}H_{44}FN_3S$	69.49	9.85	9.35	69.30	10.00	9.47
4-Chlorobenzaldehyde	85	$C_{26}H_{44}ClN_3S$	66.98	9.52	9.02	66.62	9.98	9.18
2,4-Dichlorobenzaldehyde	72-75	$C_{26}H_{43}Cl_2N_3S$	62.38	8.66	8.39	62.79	9.11	8.42
3,4-Dichlorobenzaldehyde	166-168	$C_{26}H_{43}Cl_2N_3S$	62.38	8.66	8.39	62.54	8.90	8.72
2-Nitrobenzaldehyde	138-140	$C_{26}H_{44}N_4O_2S$	65.50	9.30	11.75	65.29	9.32	11.41
3-Nitrobenzaldehyde	166	$C_{26}H_{44}N_4O_2S$	65.50	9.30	11.75	65.91	9.18	11.87
4-Nitrobenzaldehyde	135	$C_{26}H_{44}N_4O_2S$	65.50	9.30	11.75	65.02	9.11	11.98
4-Tolualdehyde	70-73	$C_{27}H_{47}N_3S$	72.75	10.63	9.43	72.82	10.83	9.24
α-Naphthaldehyde	115-17	$C_{30}H_{47}N_3S$	74.79	9.83	8.73	74.85	9.90	8.82
Salicylaldehyde	106	$C_{25}H_{43}N_3OS$	69.74	10.13	9.39	69.42	9.94	9.07
2-Methoxybenzaldehyde	56	$C_{27}H_{47}N_3OS$	70.69	9.67	9.16	70.45	9.51	9.20
Anisaldehyde	60-62	$C_{27}H_{47}N_3OS$	70.69	9.67	9.16	70.53	9.54	9.18
2-Ethoxybenzaldehyde	95	$C_{28}H_{49}N_3OS$	70.68	10.38	8.85	71.00	10.00	8.50
2-Methoxy-5-t-butylbenzaldehyde	57	$C_{31}H_{55}N_3OS$	71.89	10.71	8.12	71.25	11.03	8.21
5-Chlorosalicylaldehyde	93	$C_{26}H_{44}ClN_3OS$	64.76	9.20	8.72	64.80	9.04	8.86
5-Bromosalicylaldehyde	72	$C_{26}H_{44}BrN_3OS$	59.29	8.42	7.98	59.00	7.99	8.40
5-Nitrosalicylaldehyde	88-90	$C_{26}H_{44}N_4O_3S$	63.38	9.00	11.37	63.02	8.74	11.30
2-Hydroxynaphthaldehyde	66-68	$C_{30}H_{47}N_3OS$	72.38	9.52	8.43	72.57	9.32	8.67
2,4-Di-t-butyl-5-methoxybenzaldehyde	65-67	$C_{35}H_{63}N_3OS$	73.24	11.06	7.32	73.73	11.12	6.90
β-Resorcaldehyde	76	$C_{26}H_{45}N_3O_2S$	67.34	9.78	9.08	66.98	9.70	9.55
2,5-Dihydroxybenzaldehyde	77-78	$C_{26}H_{45}N_3O_2S$	67.34	9.78	9.08	67.12	9.61	9.14
2,3-Dimethoxybenzaldehyde	85	$C_{28}H_{49}N_3O_2S$	68.38	10.05	8.54	68.33	10.29	8.90
3,4-Dimethoxybenzaldehyde	68-70	$C_{28}H_{49}N_3O_2S$	68.38	10.05	8.54	68.54	10.12	8.80
3-Ethoxy-4-hydroxybenzaldehyde	75	$C_{28}H_{49}N_3O_2S$	68.38	10.05	8.54	68.45	10.02	8.53
5-Iodovanillin	137-139	$C_{27}H_{46}IN_3O_2S$	53.72	7.68	6.96	53.35	7.60	6.72
Phthalaldehydic acid	103	$C_{27}H_{44}N_3O_2S$	68.16	9.54	8.83	68.60	9.79	8.87
4-Cyanobenzaldehyde	112-114	$C_{27}H_{44}N_4S$	70.99	9.71	12.29	71.11	10.03	12.61
4-Dimethylaminobenzaldehyde	70	$C_{28}H_{50}N_4S$	70.82	10.62	11.84	70.41	10.39	11.94
4-Acetaminobenzaldehyde	85-87	$C_{28}H_{48}N_4OS$	68.80	9.90	11.47	68.52	10.01	11.40
Piperonal	185-187	$C_{27}H_{44}N_3O_2S$	68.18	9.53	8.83	67.95	9.11	9.15
4-Pyridinecarboxaldehyde	92	$C_{25}H_{44}N_4S$	69.39	10.25	12.95	69.40	10.30	13.23

[a] All melting points are uncorrected and were determined on a Fisher-Johns melting point apparatus.

(0.1 mole) in ethanol (100 ml.). The mixture was then refluxed for 1 hr. on a hot plate. In numerous instances immediate reaction took place as seen by the formation of a solid. The mixture was allowed to cool to room temperature and the reaction product filtered, washed with petroleum ether, and dried. The product was purified by recrystallizing twice from 60% ethanol. The compounds appeared as shiny crystals with yields from 80 to 95%. Table I gives a summary of the aldehydes used and a comparison of the analytical results with the calculated composition.

Biological Studies.—Acute toxicity studies for the compounds were performed in the DBA strain of mice, as maintained at the National Institutes of Health, Bethesda, Md. The chemicals were suspended in 0.25% Methocel (Dow methyl cellulose) so that the dose per 20 g. mouse was contained in 0.25 ml. for intraperitoneal injection and the results judged by 72-hr. survival. Due to the low solubility of the compounds, 2 g./kg. was the highest dose that could be injected. This amount of the various preparations was tolerated by all the mice.

Antitumor Studies.—The compounds were tested for antitumor activity in the three-tumor (Sarcoma 180, Adenocarcinoma 755, and Leukemia 1210) mouse screening program by screeners under contract to the Cancer Chemotherapy National Service Center. The testing procedures employed have been described previously.[9] Among the 34 compounds, 6 were found to exhibit significant inhibition in preliminary tests. Two of these, 4-cyanobenzaldehyde-4-octadecyl-3-thiosemicarbazone and 2,4-dichlorobenzaldehyde-4-octadecyl-3-thiosemicarbazone, showed good inhibition against the S-180 mouse tumor system with the latter compound showing confirmed activity. It was not active in the Ca-755 or L-1210 mouse tumor systems. Three preparations, namely,
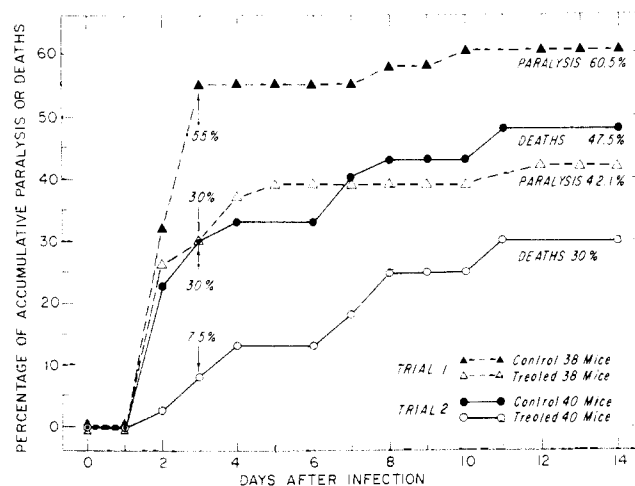
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Fig. 1.—Effect of 2,4-dichlorobenzaldehyde-4-octadecyl-3-thiosemicarbazone on experimental poliomyelitis (type 1, in mice.

2,4-di-t-butyl-5-methoxybenzaldehyde-4-octadecyl-3-thiosemicarbazone, 4-dimethylaminobenzaldehyde-4-octadecyl-3-thiosemicarbazone, and 3,4-dichlorobenzaldehyde-4-octadecyl-3-thiosemicarbazone showed inhibition against Ca-755 (testing incomplete) and one compound, valeraldehyde-4-octadecyl-3-thiosemicarbazone, possessed antitumor activity against L-1210. Table II lists the antitumor testing data for the six compounds, supplied by the Cancer Chemotherapy National Service Center.

Table II
Antitumor Activity of Certain Octadecylthiosemicarbazones Against Sarcoma 180, Adenocarcinoma 755, and Leukemia 1210

Derivative	Dose, mg./kg.	Survivors	Change in weight, g., test/control	Tumor wt., mg., test/control	T/C, %
Sarcoma 180					
2,4-Dichlorobenzaldehyde	350	3/6	−04.0/−00.2	146/863	Toxic
	175	6/6	−03.7/00.1	377/1109	33
	175	5/6	−04.7/−00.4	218/665	32
	175	5/6	−02.3/00.1	415/905	45
	175	6/6	−03.5/−00.3	195/764	25
	130	4/6	−05.1/00.8	233/913	25
	130	6/6	−02.8/00.2	435/1002	43
	130	4/6	−03.8/−00.2	336/943	35
	90	6/6	−03.8/−00.2	430/943	45
	60	5/6	−03.1/−00.2	425/943	45
4-Cyanobenzaldehyde	500	6/6	01.1/−00.6	538/1227	43
	250	6/7	−04.7/−03.7	545/1209	45
Adenocarcinoma 755					
3,4-Dichlorobenzaldehyde	225	9/10	−01.2/01.4	421/1493	28
	225	10/10	−01.6/02.4	1067/1587	67
2,4-Di-t-butyl-5-methoxybenzaldehyde	450	7/10	−05.1/00.5	368/964	38
4-Dimethylaminobenzaldehyde	175	7/10	−01.6/01.2	557/1075	51
	175	7/10	−01.9/01.6	420/1294	32
	175	10/10	−04.2/−01.1	536/1057	50
	175	5/10	00.3/02.2	565/1086	53
	175	5/10	−02.4/00.2	273/946	28
	175	7/10	−00.7/00.2	850/1079	78
Leukemia 1210					
Valeraldehyde	450	6/6	−01.9/01.5	10.3/7.9	1.30
	450	6/6	−00.7/02.7	11.6/9.2	1.26
	675	6/6	−01.9/01.7	9.1/10.1	0.90
	450	6/6	−01.5/01.7	9.6/10.1	0.95
	300	6/6	−00.6/01.7	10.5/10.1	1.03
	200	6/6	−00.2/01.7	9.5/10.1	0.94

In the light of the results with the 2,4-dichlorobenzaldehyde derivative, a test of its effectiveness in clinical trials is indicated.

Antiviral Activity of 2,4-Dichlorobenzaldehyde-4-octadecyl-3-thiosemicarbazone.[10]—Since this compound has shown confirmed activity in the S-180 mouse tumor system, it was decided to test the substance for antiviral activity before undertaking a test of all the compounds in the series. Preliminary experiments for antiviral activity of the compound against experimental polio-

myelitis in mice were carried out as follows. A group of 38 Swiss white mice were fed the drug for 8 days at a daily dose of 0.5 g./kg. of body weight. On the second day of feeding, these mice along with 38 untreated controls were challenged intracerebrally with $10^{5.5}$ TCID$_{50}$ of Type I poliovirus, L Sa strain. The paralytic rates were 42 and 60.5% in the treated and control groups, respectively. The same type of experiment was repeated but the treatment with the drug started 5 days before virus infection and stopped 2 days afterwards. Here the death rate was 30% for the treated and 47.5% for the controls. In both trials, the difference between the treated and controls seemed to be more marked in the early course of infection (Fig. 1, third and fourth day).

(10) Dr. Y. T. Chang of the Laboratory of Pharmacology and Toxicology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, studied the compound for its effect on rat leprosy in mice and found it to be inactive as a leprocidal agent.

New Compounds

Nucleosides. IV.
1-(2-Deoxy-β-D-lyxofuranosyl)-5-iodouracil[1]

Jerome P. Horwitz, Jonathan Chua, Michael Noel, and Margaret A. DaRooge

The Rollin H. Stevens Memorial Laboratory, Detroit Institute of Cancer Research, Detroit, Michigan 48201

Recently, syntheses were described for the conversion of thymidine[2,3] and 5-fluoro-2'-deoxyuridine[3] to the corresponding 2-deoxylyxosyl (-xylosyl) epimers *via* 2,3'-anhydronucleoside intermediates. In view of the marked antiviral activity[4-8] of 5-iodo-2'-deoxyuridine, it appeared of interest to extend these methods to the synthesis of 1-(2-deoxy-β-D-lyxofuranosyl)-5-iodouracil (IV).

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