TABLE I

Method	Catalyst	% composition[b] I	III	IV	V
A	*a*	7.05	1.76	85.44	5.73
B	*a*	9.34	4.61	76.69	9.34
B	*b*	1.0	7.64	2.19[i]	67.96
B	*c*	<1.0	7.1	<3.0[j]	35.2
B	*d*, *e*			*k*	>90.
	f	±1.0	4.68	2.67[l]	67.52
B	*g*				

[a] 5% palladium on carbon. [b] 5% rhodium on carbon (2.6 g./0.1 mole of V). [c] 5% rhodium on carbon (2.6 g./0.05 mole of V). [d] Platinum oxide (0.26 g./0.1 mole of V). [e] A reaction was interrupted at an early stage to determine whether the unknown was formed. [f] Uptake complete for 0.1 mole of hydrogen. [g] No uptake was observed using Raney nickel (3–4 g./0.1 mole of V) following B or in alcohol. [h] The compounds are listed in order of their place on the chromatograms run on the Aerograph machine, Model A-90-C. The samples were run on a 3-m. × 0.625-cm. (o.d.) coiled column of 18% silicone L46 and 2% Carbowax 20M on acid-washed 80–100 mesh Chromosorb W. The column was operated at 220°, the injector at 250°. Helium was used as the carrier gas at an inlet pressure of 1.35 kg./cm.². II, 2-amino-1-phenylpropane; III, 2-amino-1-(4-chlorophenyl) propane; IV, 2-(N-benzylamino)-1-phenylpropane; V, 2-(N-benzylamino)-1-(4-chlorophenyl)propane. [i] An unknown compound corresponding to about 20–21% of the product submitted was observed in the chromatogram between IV and V. [j] 41–42% of the same unknown. [k] 3–4% of unknown. [l] About 24% of the unknown. So far attempts to isolate it in a preparative chromatographic unit have failed.

of 98.5–99.5% purity. The reduction must be watched, however. In one experiment, about 80% of the dehalogenated product IV was obtained[7] when the reaction was allowed to run too long.

Anal. Calcd. for $C_{16}H_{18}ClN$: C, 73.97; H, 6.98; N, 5.39. Found: C, 74.39; H, 6.94; N, 5.39.

Hydrochloride Salt.—The salt may be recrystallized from absolute alcohol or hot water. It first melted at 193–195° but appeared to retain solvent of crystallization. After several days' drying, the melting point was raised to 209–210.5°.

Anal. Calcd. for $C_{16}H_{19}Cl_2N$: C, 64.90; H, 6.46; N, 4.73. Found: C, 64.89; H, 6.31; N, 4.60.

Attempted Debenzylation of V to III. A.—To a solution of 0.36 mole of dry hydrogen chloride in 125 ml. of ethyl alcohol was added 46.71 g. (0.18 mole) of V. Heavy precipitation occurred. The addition of 75 ml. of water did not cause the precipitate to redissolve. Six grams of 5% palladium on carbon was added and the suspension subjected to hydrogenation under 2 kg./cm.² pressure. When uptake of 0.18 mole of hydrogen was complete, the material was filtered and washed with 50% aqueous alcohol until all the insoluble material was dissolved. The solution was then concentrated to dryness under reduced pressure. It was treated with dry benzene and reconcentrated several times to remove any adhering moisture. The dried product weighed 37.7 g., m.p. 187°. After recrystallization from hot absolute ethyl alcohol, it melted at 199–200°.[8]

Anal. Found: C, 72.96; H, 7.65; N, 5.31. Since the halogen contained in the compound is ionic, its values are in close agreement with the calculated values of the hydrochloride salt of IV, $C_{16}H_{20}ClN$: C, 73.36; H, 7.70; N, 5.35.

B.—In another experiment, 25.95 g. (0.1 mole) of V was dissolved in 100 ml. of glacial acetic acid. Hydrogenation was carried out in the presence of 2.6 g. of 5% palladium on carbon under 2–3 kg./cm.² pressure. At the end of 3.5 hr., uptake of 0.1 mole of hydrogen was complete. The solution was filtered from the catalyst and concentrated under reduced pressure. The residue was treated with water and excess sodium hydroxide. The cooled mixture was extracted with either ether or benzene. The extract was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was fractionated. A constant boiling main fraction was collected at 150–153° (3 mm.), $n^{25}D$ 1.5533.[8] The results of elemental analysis indicate that the compound is indeed IV.

Anal. Calcd. for $C_{16}H_{19}N$: C, 85.29; H, 8.51; N, 6.22. Found: C, 85.37; H, 8.61; N, 6.29.

A study was made of the attempted debenzylation of V with other catalysts. Following the procedure described in B, the bases before distillation were submitted for vapor phase chromatography. The results are shown in Table I.

Pharmacology.—Compound V, 2-(N-benzylamino)-1-(4-chlorophenyl)propane, as hydrochloride salt, was given orally (in suspension) to three series of 4 rats each at dose levels of 0.011, 0.022, and 0.044 mmole/kg. The food intake was measured in 2 hr. and compared with the controls. Inhibition was 14.3, 28, and 40%, respectively. No central nervous system stimulation was observed at any dose level.

(7) R. Baltzly, *J. Am. Chem. Soc.*, **74**, 4586 (1952), in describing the preparation and properties of platinized charcoal, says it is quite inactive in dehalogenations. The commercial catalyst used in this study may be much more active.

(8) H. Temmler, French Patent 844,228 (July, 1939), gives 170–172° (10 mm.); E. H. Woodruff, J. P. Lambooy, and W. E. Burt, *J. Am. Chem. Soc.*, **62**, 922 (1940), describe the b.p. of N-benzyl-1-methyl-2-phenethylamine as 178° (13 mm.), and the m.p. of the hydrochloride salt as 198–199°.

The Identity of an Alleged Hypocholesteremic Agent Isolated from Bovine Pituitary

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In our continuing search for agents affecting lipid metabolism, we have investigated the nature of a cholesterol-lowering agent reported to be present in both the posterior and anterior lobes of the pituitary gland.[1] This agent has been isolated and a number of physical properties have been determined.[1b]

We have approached the problem by conducting an examination of the nonprotein fractions of the posterior and anterior lobes of beef pituitary. The minced lobes were extracted with acetone at room temperature as described by Wachtel[1b] and the acetone-insoluble fraction was discarded. Chromatography of the extract from the anterior lobe yielded I, m.p. 148–150°, and II, m.p. 84–86°. Chromatography of the extract from the anterior lobe yielded I and II as well as an oily fraction (III), $\nu_{max}^{CHCl_3}$ 1755 cm.$^{-1}$, which had not been detected in the extract from the posterior lobe.

Fraction I was shown to be identical with cholesterol by melting point, mixture melting point, rotation, and infrared spectrum. These parameters, in turn, are virtually identical with those reported for Wachtel's pituitary extract.[1b] It appears that Wachtel's extract is, in fact, cholesterol.

We considered that the dramatic cholesterol-lowering activity reported by Wachtel[1b] might have been due to a contaminant in his cholesterol fraction. We thus examined the biological properties of fractions II and III. The compounds were administered subcutaneously for 7 days to Albino rats of both sexes at a dose level of 25 mg./kg. Both compounds failed to cause a change in the following biochemical parameters determined in the serum: total sterol, glucose, sodium, potassium, uric acid, total nitrogen, and phospholipid. No significant

(1) (a) H. K. Wachtel, *Nature*, **163**, 254 (1949); (b) H. K. Wachtel, U. S. Patent 3,034,963 (May 15, 1962); (c) H. K. Wachtel in "Drugs Affecting Lipid Metabolism," S. Garattini and R. Paoletti, Ed., Elsevier Publishing Co., New York, N. Y., 1961, p. 201.

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-acylsphingosine 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 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%).

(2) Courtesy of Canada Packers Limited, Montreal.

(3) Courtesy of Dr. E. Greselin and Staff, Pathology Department.

Long-Chain Thiosemicarbazones as Potential Anticancer and Antiviral Agents

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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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