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THE BIOLOGICAL ACTIVITY OF SOME GUANYLHYDRAZONES AND THIOSEMICARBAZONES OF ALIPHATIC CARBONYL COMPOUNDS*

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ABSTRACT

Propanal thiosemicarbazone (**1a**) showed activity in preventing anaphylactic shock in a mouse test-system; it also had some activity in stunting the growth of *Botrytis allii*. Hexanal thiosemicarbazone (**1b**) was active in the *Botrytis allii* test-system, and citral thiosemicarbazone (**2**) and citral guanylhydrazone nitrate (**3**) showed some activity in the same test-system. Heptanal guanylhydrazone nitrate (**4**) had some antibacterial activity against *Staphylococcus aureus*, and D-*threo*-pentosulose bis(thiosemicarbazone) (**5**) prevented anaphylactic shock in the mouse test-system. D-*glycero*-Tetrosulose bis(thiosemicarbazone) (**6**), D-*lyxo*-hexosulose bis(guanylhydrazone) nitrate (**7**), D-*galacto*-heptosulose bis(thiosemicarbazone) (**8**), and D-*galacto*-heptosulose bis(guanylhydrazone) sulfate (**9**) showed some activity in stunting the growth of *Botrytis allii*. The copper chelate (**10a**) of D-*arabino*-hexosulose bis(thiosemicarbazone), and the copper (**11a**) and palladium (**11b**) chelates of 6-deoxy-L-*arabino*-hexosulose bis(thiosemicarbazone) showed antitumor activity in the KB cell-culture test-system. The palladium chelate **11b** also showed some activity in the leukemia P-388 mouse test-system.

INTRODUCTION

Freedlander and F. A. French[1] showed that the bis(guanylhydrazones) of glyoxal and pyruvaldehyde (methylglyoxal), as the sulfates or other appropriate salts, markedly prolong the lifespan of leukemic mice. Despite its toxicity, methylglyoxal bis(guanylhydrazone) has been used in the clinic as an antileukemia drug. French and Freedlander[2] also reported that the bis(thiosemicarbazones) of glyoxal and methylglyoxal have antitumor activity in animal test-systems, and Petering and Van Giessen[3] showed that 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone) (KTS) has pronounced anticancer activity in animal test-systems. Although KTS has

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a higher chemotherapeutic index than methylglyoxal bis(guanylhydrazone), a clinical, pharmacological trial conducted on KTS proved disappointing[4].

Craig and co-workers[5] prepared a series of phthalimido aldehydes as potential antineoplastic compounds; a number of these aldehydes showed antitumor activity in the Ehrlich ascites carcinoma test-system. The thiosemicarbazones, phenylsemicarbazones, (2,4-dinitrophenyl)semicarbazones, phenylhydrazones, (2,4-dinitrophenyl)hydrazones, phenylsulfonylhydrazones, *p*-tolylsulfonylhydrazones, and isonicotinoylhydrazones of the phthalimido aldehydes were also tested in the Ehrlich ascites carcinoma test-system. The hydrazones were shown to be considerably more active than the free aldehydes, and it was found that the thiosemicarbazones and isonicotinoylhydrazones were less toxic than the other hydrazones tested.

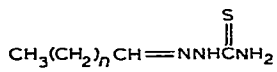
In addition to showing antitumor activity, thiosemicarbazones prepared from a variety of carbonyl compounds are known to possess tuberculostatic activity[6], antimalarial activity[7], antiviral activity[8], antifungal activity[9], and antifertility activity for screw-worm flies[9]. We have prepared a series of thiosemicarbazones and guanylhydrazones from aliphatic carbonyl compounds, and now report the results of biological screening thereof.

RESULTS AND DISCUSSION

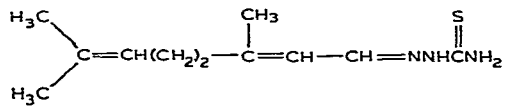
There have been reports[5,10] that propanal (propionaldehyde), heptanal, and citral have anticancer activity in animal test-systems. Because hydrazones often have antitumor activity greater than that of the parent aldehydes, we prepared the thiosemicarbazones and guanylhydrazone nitrates of the aforementioned aldehydes as potential antineoplastic compounds. We also prepared the thiosemicarbazone of hexanal, as this compound is known to have some antimicrobial activity[11]. Our compounds were tested in the leukemia L-1210 mouse test-system by the National Cancer Institute, Bethesda, Md., U. S. A. All of the compounds were inactive *vs.* leukemia L-1210.

Our compounds were also screened for biological activity by the Pharmaceuticals Division of Imperial Chemical Industries, Ltd., Macclesfield, England. It was found that propanal thiosemicarbazone (**1a**) was active in preventing anaphylactic shock in a mouse test-system; this test-system is used to detect potential immunosuppressive agents[12]. Compound **1a** also had some activity in stunting the growth of *Botrytis allii*, a fungus that causes onion-root rot; this test-system is used to detect potential antifungal agents. Hexanal thiosemicarbazone (**1b**), citral thiosemicarbazone (**2**), and citral guanylhydrazone nitrate (**3**) showed some activity in the same test-system. Heptanal guanylhydrazone nitrate (**4**) was found active as an antibacterial substance in the *Staphylococcus aureus*, test-system *in vitro*, at 100 μg/ml.

French and Freedlander[13] synthesized several bis(thiosemicarbazones) and bis(guanylhydrazone) sulfates of aldosuloses as potential anticancer compounds; all were inactive in the leukemia L-1210 mouse test-system. These authors[13] did not describe the method used for synthesis of the hydrazones. Wolfrom *et al.*[14] synthesized



1(a, $n=1$)
1(b, $n=4$)
1(c, $n=5$)



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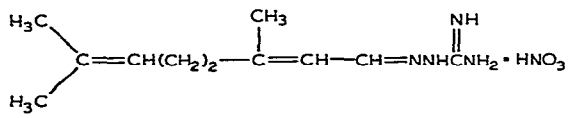

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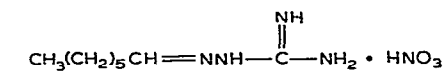

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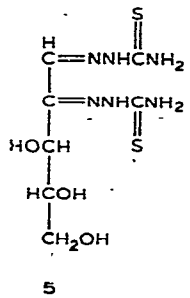
some bis(guanylhydrazone) sulfates of aldosuloses as potential antitumor compounds; they prepared the aldosuloses by Emil Fischer's method of decomposing osazones with concentrated hydrochloric acid[15].

Hamilton and Smith[16] pointed out that Weidenhagen's method[17] of oxidizing aldoses with copper acetate in methanol is possibly the most convenient procedure for preparing aldosuloses. We used Weidenhagen's method to oxidize D-glucose, D-xylose, L-rhamnose, D-*glycero*-L-*manno*-heptose, D-erythrose, and L-erythrose to the corresponding aldosuloses, which were readily converted into bis(thiosemicarbazones) and bis(guanylhydrazone) sulfates or nitrates. Some difficulty was encountered with D-galactose; the aldosulose prepared from it did not yield a crystalline bis(thiosemicarbazone) or bis(guanylhydrazone) sulfate. However, a crystalline bis(guanylhydrazone) nitrate was obtained from D-*lyxo*-hexosulose. Hirst and co-workers[18] oxidized L-ascorbic acid to dehydro-L-ascorbic acid with iodine, and the osazone of dehydro-L-ascorbic acid was readily obtained. We were unable to prepare the bis(thiosemicarbazone) of dehydro-L-ascorbic acid by Hirst's method, but the bis(4-methylthiosemicarbazone) of dehydro-L-ascorbic acid was obtained.

The hydrazones were screened for antitumor activity by the National Cancer Institute; all of them were inactive *vs.* leukemia L-1210. The Pharmaceuticals Division of Imperial Chemical Industries Ltd. found that D-*threo*-pentosulose bis(thiosemicarbazone) (**5**) has activity in preventing anaphylactic shock in the mouse test-system, and that D-*glycero*-tetrosulose bis(thiosemicarbazone) (**6**), D-*lyxo*-hexosulose bis(guanylhydrazone) nitrate (**7**), D-*galacto*-heptosulose bis(thiosemicarbazone) (**8**), and D-*galacto*-heptosulose bis(guanylhydrazone) sulfate (**9**) have some activity in stunting the growth of *Botrytis allii*.

Szent-Györgyi[19] proposed, for the control of animal cell-division, a mechanism in which an α-ketoaldehyde, possibly methylglyoxal, plays a prominent role. Szent-Györgyi[20] found difficulty in using carbonyl compounds as anticancer agents, because of the problem of maintaining a steady concentration of the carcinostatic agent in the animal body; when a small dose was administered, the agent was readily excreted, but large doses killed the animals. The biological activity shown by thio-



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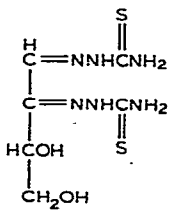

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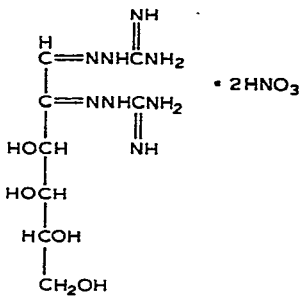

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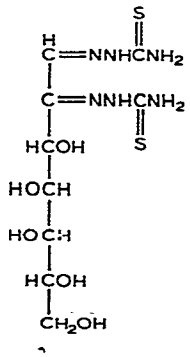

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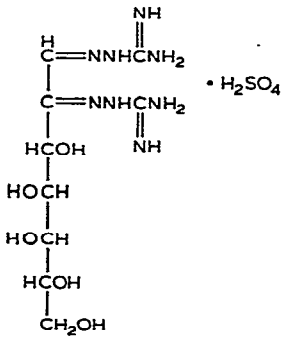

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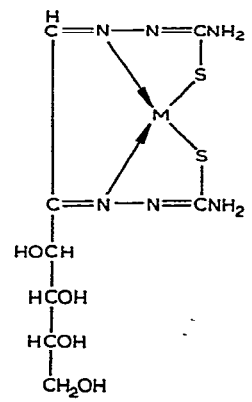
semicarbazones and guanylhydrazones may be due to their slow hydrolysis to the active carbonyl compounds.

Another mode of action of the hydrazones may be related to their ability to chelate metals. Petering and co-workers[21] reported that the cytotoxicity of KTS is enhanced *in vitro* by the presence of cupric ions. The antitumor activity in rats bearing Walker 256 carcinoma was also enhanced by cupric ions. Mihich and Mulhern[22] showed that the cupric chelate of KTS is more active than the free ligand as an anticancer agent in the sarcoma 180 ascites test-system; they considered that the activity of KTS is associated with trace-metal chelation. Booth and Sartorelli[23] studied the effect of the cupric chelate of KTS on intact, sarcoma 180 cells, and found that copper is bound intracellularly.

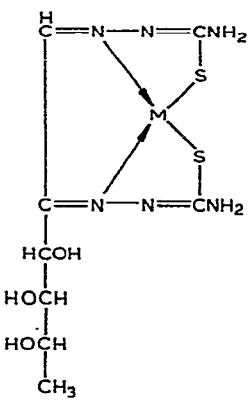
Consequently, the copper, nickel, palladium, and platinum chelates of D-*arabino*-hexosulose bis(thiosemicarbazone) were prepared; the probable structure of these chelates is given by formula **10**. These compounds were screened for antitumor activity by the National Cancer Institute; they were inactive *vs.* leukemia L-1210. In the KB cell-culture test-system, the cupric chelate (**10a**) was active (ED_{50} 2.8 μg/ml). As D-*arabino*-hexosulose bis(thiosemicarbazone) showed no cytotoxicity (ED_{50} 100 μg/ml) in the KB cell-culture test-system, our results indicate that cupric ions may be important in the antitumor activity shown by hydrazones; this conclusion agrees

with the results of Petering[21], Mihich[22], Sartorelli[23], and their collaborators. The palladium chelate **10c** (ED_{50} 8.6 μg/ml) and the nickel chelate **10b** (ED_{50} 29 μg/ml) showed some cytotoxicity in the KB cell-culture test-system.

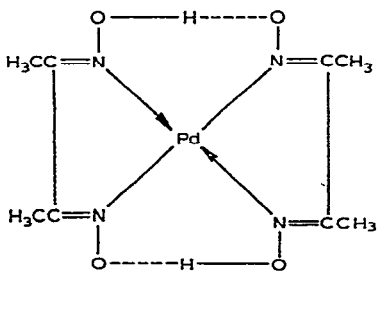
The copper and palladium chelates of 6-deoxy-L-*arabino*-hexosulose bis(thiosemicarbazone) were also prepared; their probable structure is given by formula **11**. The National Cancer Institute reported that both chelates were active in the KB cell-culture test. The copper chelate (**11a**) had an ED_{50} value of 1.9 μg/ml, and the palladium chelate (**11b**), an ED_{50} value of 2.0 μg/ml. The National Cancer Institute also found that the palladium chelate (**11b**) had some activity (test/control = 131/100 at a dose level of 100 mg/kg) in the leukemia P-388 mouse test-system. As the well known palladium chelate (**12**) of dimethylglyoxime is also active (ED_{50} 0.93 μg/ml) in the KB cell-culture test[24], it would seem that palladium, like copper, can "poison" tumor cells.



10 (a, M = Cu)
(b, M = Ni)
(c, M = Pd)
(d, M = Pt)



11 (a, M = Cu)
b, M = Pd)



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EXPERIMENTAL

General. — Propanal thiosemicarbazone (**1a**), hexanal thiosemicarbazone (**1b**), heptanal thiosemicarbazone (**1c**), and citral thiosemicarbazone (**2**) were prepared according to methods published in the literature[25,26]. D-Erythrose was obtained by oxidation of D-glucose with lead tetraacetate,[27] and L-erythrose by Ruff degradation[28,29] of calcium L-arabinonate. D-*glycero*-L-*manno*-Heptose was obtained through the courtesy of Dr. Nelson K. Richtmyer. Unless otherwise stated, melting points (m.p.) are uncorrected, and infrared (i.r.) spectra were recorded, for potassium bromide disks, with a Perkin–Elmer "Infracord" spectrophotometer. Microanalyses were performed by Dr. W. C. Alford and associates at the National Institutes of

Health, Bethesda, Md., U. S. A., and by Dr. R. D. MacDonald, University of Melbourne, Australia.

Propanal guanylhydrazone nitrate. — Aminoguanidine hydrogen carbonate (4.3 g) was suspended in water (25 ml), and the pH was adjusted to 5 with 5M nitric acid. Propanal (2.0 g) was added with vigorous stirring, and the mixture was kept at 5°. The product was collected by filtration, and recrystallized from water; the white needles (5.0 g) had m.p. 108°.

Anal. Calc. for $C_4H_{11}N_5O_3$: C, 27.12; H, 6.26; N, 39.53. Found: C, 27.09; H, 6.06; N, 39.52.

Heptanal guanylhydrazone nitrate (**4**). — Heptanal (1.7 g) was treated with aminoguanidine hydrogen carbonate (2.0 g) in aqueous nitric acid, as described for the propanal derivative. Recrystallization of the product from M nitric acid afforded white crystals (2.4 g), m.p. 83°.

Anal. Calc. for $C_8H_{19}N_5O_3$: C, 41.19; H, 8.21; N, 30.02. Found: C, 41.31; H, 8.17; N, 29.81.

Citral guanylhydrazone nitrate (**3**). — Aminoguanidine hydrogen carbonate (8.7 g) was suspended in water (30 ml), and the pH was adjusted to 5 with 5M nitric acid; this solution was treated with citral (9.7 g) in methanol (30 ml), and the mixture was stirred for 0.5 h. The crude product separated at 5°; it was filtered off, dried, and extracted for 3 h with ether in a Soxhlet extractor (to remove unreacted citral). Recrystallization from aqueous methanol afforded **3** as white crystals (5.0 g), m.p. 115°.

Anal. Calc. for $C_{11}H_{21}N_5O_3$: C, 48.69; H, 7.80; N, 25.81. Found: C, 48.52; H, 7.56; N, 25.85.

D-threo-*Pentosulose bis(thiosemicarbazone)* (**5**). — D-Xylose (12.0 g) was oxidized with cupric acetate monohydrate (60 g) in water (30 ml) plus methanol (750 ml) by Weidenhagen's method[16,17]. After filtering off cuprous oxide, removing cupric ions as the sulfide, and concentrating the solution *in vacuo*, the aldosulose was obtained as a yellow syrup; this was dissolved in methanol, the suspension filtered, and the filtrate evaporated to a white foam (10.5 g). Thiosemicarbazide (13.0 g) in water (150 ml) was added to a solution of this D-*threo*-pentosulose in water (15 ml) containing glacial acetic acid (4 ml). On heating the mixture for 1 h on a steam-bath, the crude, yellow product (6.2 g) precipitated. After four recrystallizations from hot water, **5** was obtained as yellow needles, m.p. 225° (dec.); i.r. bands at 3400 (OH and NH), 1500 (S=C–N), and 1080 cm^{-1} (C=S).

Anal. Calc. for $C_7H_{14}N_6O_3S_2$: C, 28.56; H, 4.79; N, 28.55; S, 21.79. Found: C, 28.68; H, 5.03; N, 28.54; S, 21.62.

D-galacto-*Heptosulose bis(thiosemicarbazone)* (**8**). — Oxidation of D-*glycero*-L-*manno*-heptose monohydrate (10.0 g) according to Weidenhagen's method afforded D-*galacto*-heptosulose (8.0 g). The bis(thiosemicarbazone) (3.3 g) was obtained by treating D-*galacto*-heptosulose (6.6 g) with thiosemicarbazide (5.8 g) in aqueous acetic acid as just described. Recrystallization from hot water yielded yellow needles (1.5 g), m.p. 223° (dec.).

Anal. Calc. for $C_9H_{18}N_6O_5S_2$: C, 30.50; H, 5.12; N, 23.71; S, 18.09. Found: C, 30.59; H, 4.93; N, 23.51; S, 18.06.

D-galacto-*Heptosulose bis(guanylhydrazone) sulfate* (**9**). — Aminoguanidine hydrogen carbonate (6.0 g) was suspended in water (15 ml), and the pH was adjusted to 4 with 50% sulfuric acid. The resulting solution was added to D-*galacto*-heptosulose (4.6 g) in ethanol (15 ml) containing glacial acetic acid (5 ml), and the mixture was heated for 12 min on a steam-bath, and kept for 18 h at 5°; the crude product crystallized. Two recrystallizations from hot water afforded **9** as white needles (1.9 g), m.p. 220° (dec.).

Anal. Calc. for $C_9H_{22}N_8O_9S$: C, 25.84; H, 5.30; N, 26.78; S, 7.66. Found: C, 25.67; H, 5.15; N, 26.65; S, 7.48.

D-lyxo-*Hexosulose bis(guanylhydrazone) nitrate* (**7**). — D-*lyxo*-Hexosulose was obtained by oxidizing D-galactose with cupric acetate in aqueous methanol in the usual way. A solution (pH 3) of aminoguanidine hydrogen carbonate (23 g) in 2M nitric acid was added to D-*lyxo*-hexosulose (14 g). After being filtered, the mixture was kept for 20 h at room temperature. Some of the solvent was removed in a stream of air, the mixture was clarified by filtration, and the filtrate was kept for 24 h at 5°. The product that separated (6.1 g) was filtered off, washed with ether, and dried; it had m.p. 190° (dec.; preliminary softening at 140°). The solid was dissolved in water (30 ml) at 30°, and the solution was treated with decolorizing charcoal, and kept for 24 h at 5°. The product that crystallized (2.6 g) had m.p. 191–192° (dec.; preliminary softening 130–140°).

Anal. Calc. for $C_8H_{20}N_{10}O_{10}\cdot 1.5H_2O$: C, 21.67; H, 5.23; N, 31.59. Found: C, 21.88; H , 5.06; N, 31.67.

On being dried for 3 h at 100°, the crystals darkened.

Anal. Calc. for $C_8H_{20}N_{10}O_{10}$: C, 23.08; H, 4.84. Found: 22.34; H, 4.72.

D-glycero-*Tetrosulose bis(thiosemicarbazone)* (**6**). — D-Erythrose (5.8 g) was converted into D-*glycero*-tetrosulose (5.6 g) by Weidenhagen's method. Treatment of D-*glycero*-tetrosulose (5.6 g) with thiosemicarbazide (9.1 g) in aqueous acetic acid afforded the crude carbazone as yellow flakes. Three recrystallizations from hot water yielded **6** as yellow needles (1.8 g), m.p. 227–228° (dec.); $[\alpha]_D^{22}$ +43° (*c* 0.46, pyridine).

Anal. Calc. for $C_6H_{12}N_6O_2S_2$: C, 27.26; H, 4.58; N, 31.80; S, 24.26. Found: C, 27.48; H, 4.87; N, 31.43; S, 23.62.

L-glycero-*Tetrosulose bis(thiosemicarbazone)*. — L-*glycero*-Tetrosulose (3.9 g), obtained from L-erythrose (7.0 g) by oxidation, was treated with thiosemicarbazide (6.2 g) in aqueous acetic acid. After two recrystallizations from water, the product was obtained as yellow needles (0.6 g), m.p. 228° (dec.); $[\alpha]_D^{22}$ −48° (*c* 0.63, pyridine). This compound and compound **6** gave identical i.r. spectra and identical X-ray powder photographs.

Anal. Calc. for $C_6H_{12}N_6O_2S_2$: C, 27.26; H, 4.58; N, 31.80; S, 24.26. Found: C, 26.99; H, 4.76; N, 31.60; S, 24.80.

D-glycero-*Tetrosulose bis(guanylhydrazone) sulfate*. — D-*glycero*-Tetrosulose

(4.9 g) was treated with aminoguanidine hydrogen carbonate (11.5 g) in aqueous sulfuric acid in the usual way. After some of the solvent had been removed in a stream of air, the mixture was kept for 120 h at 5°, and the crude product was collected by filtration. Three recrystallizations from hot water afforded white needles (1.3 g), m.p. 209° (dec.).

Anal. Calc. for $C_6H_{16}N_8O_6S \cdot 2H_2O$: C, 19.78; H, 5.53; N, 30.76; S, 8.81. Found: C, 19.75; H, 5.44; N, 30.55; S, 8.79. Loss on drying. Calc.: 9.9. Found: 10.6.

L-glycero-*Tetrosulose bis(guanylhydrazone) sulfate.* — L-*glycero*-Tetrosulose (5.3 g) was converted into the crude hydrazone sulfate (2.3 g) as described for the D isomer. Two recrystallizations from hot water afforded white needles (0.8 g), m.p. 209° (dec.); its i.r. spectrum was almost identical with that of the D isomer.

Anal. Calc. for $C_6H_{16}N_8O_6S \cdot H_2O$: C, 20.81; H, 5.24; N, 32.36; S, 9.26. Found: C, 20.68; H, 5.61; N, 31.98; S, 8.83.

Dehydro-L-ascorbic acid bis(4-methylthiosemicarbazone). — L-Ascorbic acid (5.0 g) was oxidized with iodine according to the method of Hirst and co-workers[18]. The solution containing dehydro-L-ascorbic acid[18] was acidified with acetic acid, and treated with 4-methylthiosemicarbazide (6.0 g), and the mixture was heated for 15 min on a steam-bath. On cooling, the crude product (1.5 g) crystallized. Recrystallization from 60% aqueous methanol afforded yellow needles (1.0 g), m.p. 235° (dec.).

Anal. Calc. for $C_{10}H_{16}N_6O_4S_2$: C, 34.47; H, 4.63; N, 24.12; S, 18.41. Found: C, 34.53; H, 4.54; N, 23.90; S, 18.13.

Synthesis of metal chelates (**10**) *of* D-arabino-*hexosulose bis(thiosemicarbazone).* — D-*arabino*-Hexosulose was prepared from D-glucose according to Weidenhagen's method. The bis(thiosemicarbazone) was prepared from the hexosulose in the usual way, and, after recrystallization from hot water, it melted at 228° (dec.); lit.[30] m.p. 230–232° (dec.).

Anal. Calc. for $C_8H_{16}N_6O_4S_2$: C, 29.63; H, 4.97; N, 25.91; S, 19.77. Found: C, 29.78; H, 4.72; N, 25.67; S, 19.50.

The bis(thiosemicarbazone) (2.0 g) in hot water (75 ml) was treated with a filtered solution of cupric acetate monohydrate (1.3 g) in water (30 ml). The mixture was heated for 0.5 h on a steam-bath, and then kept for 2 h at room temperature. The red chelate **10a** (2.2 g) was collected by filtration, successively washed with water and acetone, and dried.

Anal. Calc. for $C_8H_{14}CuN_6O_4S_2$: C, 24.90; H, 3.66; Cu, 16.46; N, 21.78. Found: C, 24.56; H, 3.61; Cu, 15.33; N, 20.68.

The bis(thiosemicarbazone) (1.5 g) was treated with potassium palladous chloride (1.65 g), as described for the copper chelate, to yield **10c** as a dark-green solid (1.6 g).

Anal. Calc. for $C_8H_{14}N_6O_4PdS_2$: C, 22.39; H, 3.29; N, 19.59; Pd, 24.87. Found: C, 22.08; H, 3.41; N, 18.99; Pd, 24.55.

A dark-brown, platinum complex (**10d**) (1.1 g) was prepared from the bis(thiosemicarbazone) (0.9 g) and potassium platinous chloride (1.2 g).

Anal. Calc. for $C_8H_{14}N_6O_4PtS_2$: C, 18.56; H, 2.73; N, 16.24; Pt, 37.72. Found: C, 18.53; H, 2.89; N, 16.02; Pt, 37.27.

Nickel acetate tetrahydrate (1.7 g) reacted with the bis(thiosemicarbazone) (2.2 g) to yield a dark-brown chelate (**10b**) (2.2 g).

Anal. Calc. for $C_8H_{14}N_6NiO_4S_2$: C, 25.21; H, 3.71; N, 22.06; Ni, 15.41. Found: C, 24.48; H, 3.76; N, 20.51; Ni, 14.50.

Synthesis of metal chelates (**11**) *of 6-deoxy-*L-arabino-*hexosulose bis(thiosemicarbazone).* — L-Rhamnose (15 g) was oxidized to 6-deoxy-L-*arabino*-hexosulose (11 g), which yielded the bis(thiosemicarbazone) (2.7 g) on treatment with thiosemicarbazide. Recrystallization of the carbazone from hot water afforded yellow needles (2.5 g), m.p. 222° (dec.).

Anal. Calc. for $C_8H_{16}N_6O_3S_2$: C, 31.16; H, 5.23; N, 27.25; S, 20.80. Found: C, 30.97; H, 5.15; N, 27.12; S, 20.91.

A solution of the bis(thiosemicarbazone) (0.9 g) in hot water (75 ml) was treated with potassium palladous chloride (1.0 g) in water (30 ml). The mixture was heated for 0.5 h on a steam-bath, and cooled to room temperature. The black chelate **11b** (0.8 g) was filtered off, successively washed with water and a little acetone, and dried.

Anal. Calc. for $C_8H_{14}N_6O_3PdS_2$: C, 23.26; H, 3.42; N, 20.35; Pd, 25.83; S, 15.52. Found: C, 22.65; H, 3.42; N, 20.08; Pd, 24.48; S, 16.83.

A red, copper chelate **11a** (0.8 g) was prepared by treating the bis(thiosemicarbazone) (2.0 g) with cupric acetate monohydrate (1.4 g) in aqueous solution.

Anal. Calc. for $C_8H_{14}CuN_6O_3S_2 \cdot 2H_2O$: C, 23.67; H, 4.47; N, 20.70; S, 15.80. Found: C, 23.59; H, 3.60; N, 20.55; S, 16.30.

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