Summary

Citrus juices have been observed to give a yellow color when dried with boric acid.

This yellow color forming substance is concentrated in the citrin fraction of Szent-Györgyi.

Many flavone derivatives have been found to give the color reaction. Of non-flavone substances commonly found in plant tissues, none have so far been found to give a confusable color reaction.

The color reaction has been correlated with molecular constitution.

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[CONTRIBUTION FROM THE AVERY LABORATORY OF CHEMISTRY OF THE UNIVERSITY OF NEBRASKA]

Organoselenium Compounds. I. Diphenylselenium Dihydroxides and Diphenylselenides

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In order to investigate some of the therapeutic properties of organoselenium compounds, a series of compounds in which the effect of the valence of selenium could be studied, as well as the effect of changes in substituent groups, was prepared. This paper deals with some of the derivatives of diphenylselenium dihydroxide and diphenylselenide derived from the phenoxyalkanols, as these compounds have led to some therapeutically valuable arsenicals.

The reaction of Alquist and Nelson[2] and Kunckell[3] by which anisole and its homologs were condensed with selenium oxychloride to give *bis*-(*p*-alkyloxyphenyl)-selenium dichlorides was extended to three other ethers, β-phenoxyethanol, γ-phenoxypropanol and α-methyl-β-phenoxyethanol. The first two gave ether insoluble gums, while the last gave a crude crystalline mass. The crude products were purified by dissolving in chloroform, treatment with activated charcoal and "Celite," and reprecipitation with benzene. By means of this procedure all three crystallized in stable forms. The dichlorides hydrolyzed to the dihydroxides in the presence of sodium carbonate, all three of which were white crystalline solids.

The above dihydroxides, as well as *bis*-(4-methoxyphenyl)-selenium dihydroxide, were then nitrated using fuming nitric acid (sp. gr. 1.5) at 0° as the nitrating agent. A large excess of acid was required for complete nitration, probably due to the formation of an alcohol dinitrate of the dinitrodietherdiphenylselenium dinitrate. The gum formed when the nitration mixture was poured over ice was solidified by neutralizing the excess acid with sodium carbonate and boiling. These compounds proved to be of the formula *bis*-(3-nitro-4-R-phenyl)-selenium dihydroxide, R representing the ether group. The position of the nitro group was shown by cleaving with hydriodic acid, giving *o*-nitrophenol.

The nitro compounds were reduced catalytically to the diamines, using Raney catalyst.[4] At 40 pounds (2.67 atm.) pressure and room temperature the selenium was also reduced. The amines were of the general formula *bis*-(3-amino-4-R-phenyl) selenide.

Of theoretical interest was the extension of the reaction to another type of compound, acetanilide. The reaction was first tried in ether but it was found that the product was a double salt of acetanilide and selenium oxychloride. As the salt was soluble in chloroform, the reaction was run in that medium. After five days, a thick gum separated. This gum was hydrolyzed with sodium carbonate, giving a water insoluble white compound which, on purification, proved to be *bis*-(4-acetaminophenyl)-selenium dihydroxide. This compound was reduced to the corresponding diphenyl selenide and then hydrolyzed to the free amine. The last two compounds have been prepared by Theobald[5] from sulfonamide and selenic acid. The acetylated compound was obtained in two crystalline modifications, one melting at 216° as reported by Theobald, the other at 176°. The two forms were chemically identical.

Experimental

1. Diphenylselenium Dichlorides.—One-half mole of the aromatic ether was treated with selenium oxychloride according to the procedure of Alquist and Nelson.[2] The solvent was decanted from the gum or crystals formed and

(1) Parke, Davis and Company Fellow.

(2) F. N. Alquist and R. E. Nelson, THIS JOURNAL, **53**, 4033 (1931).

(3) Kunckell, *Ber.*, **28**, 609 (1895).

(4) M. Raney, U. S. Patent 1,628,190 (1927).

(5) E. Theobald and P. Theobald, French Patent 794,192 (1936); German Patents 631,100, 631,570, 631,571, 631,572, 632,073, 633,344 (1936).

TABLE I

	Compound	Color	M. p., °C.	% Yield	Formula	% Se Calcd.	% Se Found
1	*bis*-(4-Methoxyphenyl)-selenium dichloride	Yellow	163	84	.....	..	..
2	*bis*-(4-β-Hydroxyethoxyphenyl)-selenium dichloride	Yellow	172	74	$C_{16}H_{18}O_4Cl_2Se$	18.6	18.7
3	*bis*-(4-γ-Hydroxypropoxyphenyl)-selenium dichloride	Yellow	159	66	$C_{18}H_{22}O_4Cl_2Se$	17.5	17.5
4	*bis*-(4-β-Hydroxypropoxyphenyl)-selenium dichloride	Yellow	147	80	$C_{18}H_{22}O_4Cl_2Se$	17.5	17.4
5	*bis*-(4-Methoxyphenyl)-selenium dihydroxide	White	134	90	.....	..	..
6	*bis*-(4-β-Hydroxyethoxyphenyl)-selenium dihydroxide	White	99	78	$C_{16}H_{20}O_6Se$	20.4	20.5
7	*bis*-(4-γ-Hydroxypropoxyphenyl)-selenium dihydroxide	White	140	67	$C_{18}H_{24}O_6Se$	19.0	19.1
8	*bis*-(4-β-Hydroxypropoxyphenyl)-selenium dihydroxide	White	56	63	$C_{18}H_{24}O_6Se$	19.0	19.1
9	*bis*-(3-Nitro-4-methoxyphenyl)-selenium dihydroxide	Cream	203	95	$C_{14}H_{14}O_8N_2Se$	18.9	19.0
10	*bis*-(3-Nitro-4-β-hydroxyethoxyphenyl)-selenium dihydroxide	Cream	175	84	$C_{16}H_{18}O_{10}N_2Se$	16.6	16.5
11	*bis*-(3-Nitro-4-γ-hydroxypropoxyphenyl)-selenium dihydroxide	Yellow	117	62	$C_{18}H_{22}O_{10}N_2Se$	15.6	15.7
12	*bis*-(3-Nitro-4-β-hydroxypropoxyphenyl)-selenium dihydroxide	Yellow	128	52	$C_{18}H_{22}O_{10}N_2Se$	15.6	15.7
13	*bis*-(3-Amino-4-methoxyphenyl) selenide	White	112	>90	$C_{14}H_{16}O_2N_2Se$	23.8	23.9
14	*bis*-(3-Amino-4-β-hydroxyethoxyphenyl) selenide	White	132	>90	$C_{16}H_{20}O_4N_2Se$	22.2	22.3
15	*bis*-(3-Amino-4-γ-hydroxypropoxyphenyl) selenide	Pink	104	>90	$C_{18}H_{24}O_4N_2Se$	20.6	20.5
16	*bis*-(3-Amino-4-β-hydroxypropoxyphenyl) selenide	Brown	128	>90	$C_{18}H_{24}O_4N_2Se$	20.6	20.6
17	Dihydrochloride of 13	White	>250	78	$C_{14}H_{16}O_2N_2Se \cdot 2HCl$	20.1	20.0
18	Selenium oxychloride salt of acetanilide	White	135	80	$(C_8H_9N)_2 \cdot SeOCl_2$	18.1	18.0
19	*bis*-(4-Acetaminophenyl)-selenium dihydroxide	White	223	90	$C_{16}H_{18}O_4N_2Se$	20.6	20.5
20	*bis*-(4-Acetaminophenyl) selenide	White	176, 216	>90	$C_{16}H_{18}O_2N_2Se$	22.7	22.7
21	*bis*-(4-Aminophenyl) selenide	White	117	60	$C_{12}H_{12}N_2Se$	30.0	29.9

purified by solution in chloroform, treatment with activated charcoal and "Celite" and reprecipitation with benzene.

2. Diphenylselenium Dihydroxides.—The above dichlorides (0.2 mole) were added to an excess of sodium carbonate in water (30 g. in 200 ml.) and the mixture heated for half an hour. On cooling, if the dihydroxide crystallized, it was filtered off; if it remained an oil, the water was decanted, fresh water added, heated and cooled. This was repeated until a solid was obtained. The crude dihydroxides were recrystallized from ethyl alcohol.

3. Nitro Compounds.—The dihydroxide was added slowly with stirring to fuming nitric acid (sp. gr. 1.5) at 0° (0.025 mole to 25 ml. of acid). After the addition was complete, the stirring was continued for fifteen minutes and the nitration mixture then poured over chipped ice. Water was added and the acid neutralized with an excess of sodium carbonate. The mixture was boiled and then cooled. The nitro compounds solidified on stirring. The methoxy and γ-hydroxypropoxy compounds were recrystallized from ethyl alcohol, the β-hydroxyethoxy compound from ethyl cellosolve and the β-hydroxypropoxy compound from methyl cellosolve.

4. Amines.—The nitro compounds were suspended in ethyl alcohol (10 g. in 50 ml.), Raney nickel prepared by the method of Adkins[6] was added and the reduction carried out with electrolytic hydrogen at 40 pounds (2.67 atm.) pressure and room temperature. The solution and suspended amine were removed from the reduction container, heated to boiling and the catalyst filtered off. Most of the alcohol was removed by evaporation under reduced pressure, water and hydrochloric acid added and the solution decolorized with charcoal. The amine was then precipitated with ammonium hydroxide. Water–alcohol mixtures were used to recrystallize the amines.

5. *bis*-(3-Amino-4-methoxyphenyl) Selenide Dihydrochloride.—The corresponding amine (10 g.) was dissolved in an excess of 6 *N* hydrochloric acid and the solution evaporated until precipitation started to occur while hot. On cooling the hydrochloride crystallized out; yield 9.5 g.

6. Double Salt of Acetanilide and Selenium Oxychloride.—Eight grams of acetanilide was dissolved in 200 ml. of dry ether and an equal weight of selenium oxychloride added with shaking. A white precipitate formed almost immediately. It was purified by solution in chloroform and reprecipitation with ligroin.

7. *bis*-(4-Acetaminophenyl)-selenium Dihydroxide.—One-fifth mole of acetanilide was treated with selenium oxychloride in chloroform as in procedure 1. The excess solvent was decanted from the gum formed, an excess of sodium carbonate in water was added and the temperature raised to the boiling point. The solution was filtered hot, removing any excess acetanilide. The precipitate was dissolved in alcohol, decolorized with charcoal and reprecipitated with water.

8. *bis*-(4-Acetaminophenyl) Selenide.—Ten grams of the dihydroxide was dissolved in warm alcohol, Raney catalyst added, and the reduction carried out under the same conditions as for the reduction of nitro compounds. After the reduction was complete, the catalyst was removed and the solution reduced in volume, and water was added, precipitating the diphenyl selenide. The product was recrystallized from a water–alcohol mixture.

9. *bis*-(4-Aminophenyl) Selenide.—Ten grams of the above product was refluxed with 100 ml. of 20% hydrochloric acid. When all the material had gone into solution, the solution was charcoaled and filtered. On cooling and making basic with sodium hydroxide, the amine precipitated.

Analytical

A modification of the method of Arne Fredga[7] was used in determining the percentage of selenium. A tenth-gram sample was digested with 5 ml. of concd. sulfuric acid until it was well charred. Fuming nitric acid was added (about 1 ml.) until the solution cleared. The excess nitric acid was boiled off, the digestion transferred to a beaker containing 50 ml. of water and the whole diluted to 150 ml. Hydrazine sulfate was added and the solution heated on a hot-plate until the precipitated selenium coagulated. The precipitate was collected in a semi-

(6) Covert and Adkins, THIS JOURNAL, **54**, 4116 (1932).

(7) A. Fredga, *Uppsala Univ. Arsskrift*, No. 5, 232 (1935).

micro sintered glass crucible containing a thin layer of asbestos. After washing well with water, alcohol and ether, the crucible was dried and weighed on a semi-micro balance. The nitric acid must not be added until the compound is well charred or polynitro compounds resisting digestion will be formed. The precision of this method of analysis is ±0.0%.

Summary

1. Three new *bis*-(4-hydroxyalkyloxyphenyl)-selenium dichlorides and their corresponding dihydroxides were prepared by the condensation of selenium oxychloride with mixed ethers.

2. The dinitro derivatives of the above compounds and the corresponding methoxy compound were obtained. These compounds were reduced catalytically to the *bis*-(3-amino-4-R-phenyl) selenide.

3. The condensation of selenium oxychloride with benzene derivatives was extended to acetanilide, resulting in *bis*-(4-acetaminophenyl)-selenium dihydroxide. On reduction, *bis*-(4-acetaminophenyl) selenide was formed. The corresponding amine also was prepared.

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Studies in the Sitosterol Complex. The Structure of α_1-Sitosterol

BY SEYMOUR BERNSTEIN[1] AND EVERETT S. WALLIS

In their experiments on α-sitosterol, Wallis and Fernholz[2] showed that this material first described as a compound by Anderson and his coworkers[3] was in reality a mixture of at least two new sterols, α_1- and α_2-sitosterol.

Besides recording the physical properties of these two new compounds and their derivatives, these investigators[2] made the following observations. Neither α_1- nor α_2-sitosterol are isomers of β- and γ-sitosterol. α_1-Sitosterol is an isomer of stigmasterol, $C_{29}H_{48}O$, and α_2-sitosterol is in all probability a homolog, $C_{30}H_{50}O$. By titration with perbenzoic acid they showed that two double bonds are present in both sterols. They give the same Liebermann color reaction. The final color is a dark blue with a reddish tint. The Salkowski reaction for both α_1- and α_2-sitosterol was found to be similar to that of ergosterol, the sulfuric acid layer becomes colored, while the chloroform stays colorless. The opposite is true for cholesterol, γ-sitosterol and stigmasterol. They also noted that both α_1- and α_2-sitosterol are precipitated by digitonin.

In this paper we wish to report the results of further experiments on α_1-sitosterol which to us seem pertinent to the elucidation of its structure. That the two double bonds in α_1-sitosterol are not conjugated was shown by an absorption spectrum study. No maxima were observed. It is also to be noted that α_1-sitosterol does not form an addition compound with maleic anhydride. Taken together these two facts indicate that this sterol cannot have its double bonds conjugated within one ring or between two adjacent rings.

The results which we obtained from a hydrogenation study on α_1-sitosterol are of special interest. From preliminary experiments we learned that only one of the two double bonds can be hydrogenated under ordinary conditions. If the hydrogenation, however, be carried out at a higher temperature (65–70°) in the presence of a small amount of concentrated hydrochloric acid, complete saturation is possible. It was further observed that the double bond originally resistant to hydrogenation can be isomerized by dry hydrogen chloride into a position which is easy to hydrogenate under ordinary conditions.

With the above facts in mind, α_1-sitosteryl acetate (I) was hydrogenated with a platinum catalyst at room temperature and atmospheric pressure. As a result, α_1-dihydrositosteryl acetate (II) was obtained. This acetate was then isomerized in a chloroform solution with dry hydrogen chloride at 0°. A new acetate, α_1-isodihydrositosteryl acetate (III), was isolated and characterized. On hydrogenation at room temperature and atmospheric pressure this compound gave a completely saturated acetate, α_1-sitostanol acetate (IV). This same substance was also obtained when α_1-sitosteryl acetate was hydrogen-

(1) Research Assistant on Special Funds from the Rockefeller Foundation.

(2) Wallis and Fernholz, THIS JOURNAL, **58**, 2446 (1936).

(3) Anderson, Shriner and Burr, *ibid.*, **48**, 2987 (1926); see also Anderson, *ibid.*, **46**, 1450 (1924).