TABLE I.

Reagent.	U. S. P.	Chrysarobin.	Chrysophanic Acid.	9-Anthrone.
5% NaOH	Deep red	Red	Red	Pink
Concd. H_2SO_4	Deep red	Brownish red	Cherry red	Yellow
Fuming HNO_3	Red brown	Red brown	Yellow	Light brown
$+NH_4OH$	Violet red	Brownish red + precipitate	Violet	Brown

It will be noted that in two of the four cases the results with chrysarobin differ decidedly with the tests given by the U. S. P., that is, with concentrated sulphuric acid and on treating the fuming nitric acid solution with ammonia. In the latter case, the monograph also states that chrysophanic acid gives a yellow color with those reagents. The production of a violet color was checked using chrysophanic acid from three sources, one sample from chrysarobin and two synthetic. The colors obtained with five samples of chrysarobin from as many different dealers gave the same colors, with the exception that a few samples showed some bluish green fluorescence in sodium hydroxide solution.

These results can only lead to one conclusion, that the tests for identity for chrysarobin given in the U. S. P. are incorrect and in need of revision. No data are as yet at hand to justify any positive suggestions.

REFERENCE.

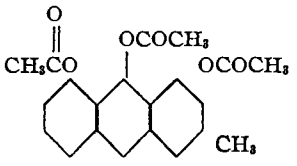
(1) Naylor and Gardner, *J. Am. Chem. Soc.*, 53 (1931), 4114.

THE PREPARATION OF CHRYSOPHANIC ACID FROM CHRYSAROBIN.*,[1]

BY JOHN H. GARDNER.

In the course of our studies on the chemistry of the natural purgatives it has become necessary to find a source from which chrysophanic acid can be readily obtained. Since chrysarobin is made up almost entirely of derivatives of chrysophanic acid and of emodin monomethyl ether, it seemed a logical material to investigate.

Several years ago, the author and Naylor (1) found that by demethylating chrysarobin with hydrobromic acid and acetylating the product, a mixture was formed from which chrysophanic acid-9-anthranol triacetate (Formula I) could be readily separated by fractional crystallization. Chrysophanic acid can be readily



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* From the Chemical Laboratory of Washington University, St. Louis. This investigation was made possible by a grant from the fund given by the Rockefeller Foundation to Washington University for research in science.

[1] Scientific Section, A. PH. A., Washington meeting, 1934.

prepared from this material, as will be shown in the Experimental Part. Unfortunately, on repeating the preparation of the anthranol triacetate using a number of samples of chrysarobin from various sources, the yields were found to be irregular and usually poor. Consequently, this method does not meet our requirements.

Hauser (2) found that chrysarobin contains a substance, ararobinol, probably a dianthrone derived from chrysophanic acid, which upon reduction yields chrysophanic acid-9-anthrone. Consequently, a new procedure was tried. Chrysarobin was first reduced with tin and hydrochloric acid with or without stannous chloride and then subjected the product to the same procedure as before. Using five different samples of chrysarobin, the anthranol triacetate was obtained in yields of 15 to 35% of the weight of chrysarobin taken. It was easily purified by crystallization from glacial acetic acid.

For the preparation of chrysophanic acid, the anthranol triacetate was oxidized with chromic acid to diacetyl chrysophanic acid and that hydrolyzed to chrysophanic acid. It was found that unless the anthranol triacetate was quite pure, it was virtually impossible to obtain the subsequent products in good states of purity.

EXPERIMENTAL.

Preparation of Chrysophanic Acid-9-Anthranol Triacetate.—A.—To a solution of 5 Gm. of chrysarobin in 200 cc. of boiling glacial acetic acid there were added 15 Gm. of 200-mesh tin and then, during three and one-half hours, 65 cc. of concd. hydrochloric acid. Boiling was continued another three and one-half hours. The mixture was then cooled and diluted with water until no more precipitate was formed. The mixture was filtered, the reduced chrysarobin being floated away from the residual tin. The crude product was sucked as dry as possible on the filter and was used without further purification.

The reduced chrysarobin was boiled under reflux with 75 cc. of glacial acetic acid and 75 cc. of 48% hydrobromic acid for fifteen hours. After cooling, the mixture was filtered and the residue dried at room temperature.

The dried residue was mixed with 5 Gm. of anhydrous sodium acetate and 55 cc. of acetic anhydride. The mixture was boiled one hour and poured onto ice. After standing 24 hours with occasional stirring, the residue was filtered out and dried at room temperature. It was then boiled with about 200 cc. of glacial acetic acid, cooled, stirred and filtered. The residue was washed with glacial acetic acid until the washings were almost colorless. Yield, 0.75 to 1.25 Gm., m. p. 228–235°.

B.—A mixture of 25 Gm. of chrysarobin, 100 Gm. of stannous chloride, 75 Gm. of 20-mesh granulated tin and 1000 cc. of glacial acetic acid was boiled under a reflux condenser with mechanical stirring for four hours. During the first two and one-half hours, 400 cc. of concentrated hydrochloric acid was added in small portions. The mixture was cooled and filtered, the reduced chrysarobin being floated away from the remaining tin.

The reduced chrysarobin was demethylated and acetylated as before, using proportionate amounts of the various reagents. Yield of chrysophanic acid-9-anthranol triacetate, 8.5 Gm. after two acetic acid treatments; m. p. 238–241° (corr.) with decomposition. McDonnell and Gardner (3) give m. p. 239.6–240°.

Diacetyl Chrysophanic Acid.—To a solution of 8.5 Gm. of chrysophanic acid-9-anthranol triacetate in 350 cc. of hot glacial acetic acid there were added 3 Gm. of chromic acid in a little water and acetic acid, in portions. The mixture was heated nearly to boiling for fifteen minutes. A small amount of insoluble tar was removed by hand. The mixture was diluted to 1 liter with water and allowed to stand over night. The product was filtered out and washed with water. Yield, 7 Gm. (89.2%), m. p. 205–206° (corr.) from alcohol. Siegrist (4) gives m. p. 208–209°. Beal and Gunton (5) give m. p. 204°.

Chrysophanic Acid.—Five grams of diacetyl chrysophanic acid were partly dissolved and partly suspended in 600 cc. of boiling ethyl alcohol. A solution of 3 Gm. of potassium hydroxide in a little water was added and the mixture boiled three hours, after which 10 cc. of concentrated

hydrochloric acid was added and the mixture allowed to stand over night. The precipitate which formed was filtered out. It was found to be a mixture of chrysophanic acid and potassium chloride. It was therefore boiled with water and the chrysophanic acid filtered out. Yield, 3.5 Gm. (95.8%), m. p. 193–194° (corr.). Naylor and Gardner (1) give m. p. 195.6–196.2°. Other investigators report slightly lower values.

SUMMARY.

A satisfactory method for the preparation of chrysophanic acid from chrysarobin has been developed.

REFERENCES.

(1) Naylor and Gardner, *J. Am. Chem. Soc.*, 53 (1931), 4114.
(2) Hauser, Dissertation, Zürich, 1924.
(3) McDonnell and Gardner, *J. Am. Chem. Soc.*, 56 (1934), 1246.
(4) Siegrist, Dissertation, Basel (1932), 49.
(5) Beal and Gunton, JOUR. A. PH. A., 11 (1922), 681.

THE BIOASSAY OF SQUILL.*[1]

BY HARRY ROSEN.

Squill, a member of the heart stimulant or digitalis group of drugs is biologically assayed by the same methods as the other members of this so-called "Digitalis series."

Many test animals and methods of procedure have been proposed and employed for bioassay and standardization purposes. Consequently a wide divergence of opinion concerning the relative merits of the respective procedures is found.

Hale (1), (2), after working with the various methods of assay, concluded that they did not give proportional results and suggested that the one-hour frog procedure was probably the most suitable.

The American Drug Manufacturers' Association (3) undertook collaborative investigations of the various assay methods and concluded that the M. L. D. frog and M. L. D. guinea pig methods were more accurate than the one-hour frog or cat methods, and that the technique involved was much simpler.

Richaud (4), studying the various methods for the assay of cardiac tonics, concluded that the guinea pig method was unsuitable for the assay of these drugs.

Eckler (5) assayed a series of preparations by the cat, guinea pig and one-hour frog methods. He concluded that the cat method was complicated, time consuming, costly and gave results varying from 33 to 123 per cent.

Rowntree and Macht (6) concluded that the cat method was more reliable than the frog method. Van Leeuwen, den Besten and van Wijngaarden (7), (8), (9) reported that the cat method was more accurate and was independent of seasonal variations as compared with the frog methods.

Wible (10) concluded that the cat and the one-hour frog methods agree within the limits of biological error.

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* Scientific Section, A. PH. A., Washington meeting, 1934.

[1] From the laboratory of Marvin R. Thompson, Professor of Pharmacology, School of Pharmacy, University of Maryland. Compiled in part from a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science, June 1933.