TINCTURE OF DIGITALIS.*

BY L. W. ROWE AND WILBUR L. SCOVILLE.

This paper is a continuation of the work reported to this Section two years ago.

In November 1931, a second series of tinctures was prepared from a defatted drug assaying 200 per cent of standard.

For Series A, 125 Gm. of the drug was extracted with 77% alcohol and 1150 cc. of tincture was obtained, this being 92% of the full yield. This percolate was then divided into five portions of 230 cc. each and numbered successively.

No. 1 was diluted to 250 cc. with 77% alcohol.

No. 2 was adjusted to a p_H of 2.93 by the addition of 1.5 cc. of hydrochloric acid, then made up to a volume of 250 cc. by the addition of 2.5 cc. of water and 16 cc. of alcohol.

No. 3, which had an initial p_H of 5.56, was adjusted to a p_H of 6.76 by the addition of sodium hydroxide solution, then made up to a volume of 250 cc. with 77% alcohol.

To No. 4 was added 2.5 cc. of glacial acetic acid, then 15 Gm. of anhydrous sodium acetate was dissolved in the liquid and the final volume was adjusted to 250 cc. by addition of alcohol.

No. 5 was treated with 50 Gm. of anhydrous sodium sulphate, the mixture being agitated frequently during one week, then filtered and the filter washed with 95% alcohol to obtain a yield of 250 cc. This assayed 78.6% of alcohol and showed a p_H of 5.41.

Series B was made from another 125 Gm. of the same drug but it was first sterilized in the following manner. The drug was mixed with 120 cc. of 95% alcohol, allowed to stand over night, then heated on a steam-bath under a reflux condenser for 20 minutes, cooled, 30 cc. of water added and well mixed, then transferred to a percolator and extracted with 77% alcohol to obtain 1150 cc. of percolate.

This was divided into five equal portions and the series made to correspond to Series A; No. 1 being diluted to 250 cc. with menstruum; No. 2 adjusted to a p_H of 3.01 with hydrochloric acid; No. 3 adjusted to a p_H of 6.70 with sodium hydroxide; No. 4 saturated with anhydrous sodium acetate (15 Gm.) and 2.5 cc. glacial acetic acid added to maintain an acid reaction; and No. 5 partially dehydrated with 50 Gm. of anhydrous sodium sulphate. The alcohol in No. 5 when finished tested 79.9%. Each was adjusted to a final volume of 250 cc.

Series C was made with 87% alcohol, without sterilization, the 1150 cc. of percolate being divided into five portions of 230 cc. each. No. 1 was diluted to 250 cc. by addition of 87% alcohol, No. 2 was adjusted to a p_H of 2.80 with hydrochloric acid, No. 3 to p_H 6.80 with sodium hydroxide, No. 4 was saturated with (10 Gm. of) anhydrous sodium acetate and kept acid by means of 2 cc. of glacial acetic acid, and No. 5 was treated with 30 Gm. of anhydrous sodium sulphate. This showed an alcohol content of 87.2% when finished.

All samples were adjusted to a final volume of 250 cc., were assayed soon after completion, then all were stored in amber bottles in a laboratory room and in diffused light.

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The second assay was made 11 months after the first.

The following table shows the stability of the samples during an eleven months' period.

Series A.	p_H.	First Assay.	Second Assay.	Series B.	p_H.	First Assay.	Second Assay.	Series C.	p_H.	First Assay.	Second Assay.
No. 1	5.38	110%	85%	No. 1	5.37	110%	90%	No. 1	5.16	100%	90%
No. 2	2.93	110	120	No. 2	3.01	110	110	No. 2	2.80	110	65
No. 3	6.76	100	90	No. 3	6.70	110	110	No. 3	6.80	150	85
No. 4	6.10	110	120	No. 4	6.07	100	120	No. 4	6.25	150	90
No. 5	5.41	110	150	No. 5	..	110	70	No. 5	..	100	80

Two lots of tincture made with 77% alcohol and stabilized with anhydrous sodium acetate and acetic acid in 1930 were again assayed with the following results:

Tinct. G. 125% on Dec. 1, 1930, 125% on May 28, 1931, 120% in November 1932.
Tinct. F. 125% on Dec. 1, 1930, 125% on May 28, 1931, 120% in November 1932.

One of us (L. W. R.) has also tested the toxicity of anhydrous sodium acetate on frogs and white mice, and reports as follows:

> "The solution used contained 6% of anhydrous sodium acetate in 70% alcohol and also 1% of acetic acid. By the one-hour frog method 0.015 cc. per Gm. was necessary to prostrate the frog, while 0.025 cc. per Gm. did not stop the heart in systole. The minimum systolic dose for Tr. Digitalis, U. S. P. X is 0.006 cc. per Gm. so that more than four times as much of the control solution failed to show any digitalis action. The minimum lethal dose for frogs is about 0.050 cc. per Gm. and then the heart stops in diastole. The solution was more toxic to white mice as a dose of 0.010 cc. per Gm. was just fatal.
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> "It could not be denied that this control solution possessed some toxicity for these small laboratory animals, the white mouse and the frog, but the toxic action was not at all like that of the digitalis glucosides, so it really should not affect the assay appreciably."

These results may be summarized as follows: The plain, unadjusted tincture shows a 15% deterioration in eleven months; from sterilized drug a 10% deterioration, and when made with 87% alcohol 10% deterioration in the same time.

When adjusted to a p_H of about 3.0 with hydrochloric acid, no deterioration is shown in the two tinctures made with 77% alcohol, but 40% deterioration appears in that made with 87% alcohol.

When adjusted to near neutrality by addition of sodium hydroxide a deterioration of 10% is shown in the first tincture, no deterioration in that made from sterilized drug and 45% deterioration in that made with 87% alcohol. In the latter case the tincture was on the alkaline side for a few moments during the adjustment proceedings.

In the tinctures saturated with anhydrous sodium acetate, the first shows no deterioration, the sterilized drug shows no deterioration and that made with 87% alcohol shows 40% deterioration. The reports on the first two each show a variation of 10 points—which suggests an experimental error. The last tincture is outside the pale of experimental error.

The treatment with anhydrous sodium sulphate for the purpose of dehydrating shows peculiar results. The first tincture indicates a gain of 36%, the second a loss of 37% and the third a loss of 20%. Furthermore the dried sodium sulphate proved to be a weak dehydrating agent in the tinctures as shown by the fact that the finished tinctures in each case contained about the same percentage of alcohol as the menstruum used. On the basis of the above results this method does not look promising.

Reviewing the results of three years' experiments the anhydrous sodium acetate treatment is the only one which has shown real and fairly consistent stability. In the (only) two samples made in 1930 the tinctures have remained practically stable for two years.

Adjustment of the p_H shows no advantage and other laboratories have concurred in that view. The use of a stronger alcoholic menstruum involves greater difficulty in extraction, and thus far does not indicate any greater stability in the tincture.

Sterilizing the drug before extracting has indicated some advantage in certain trials, none in others. The advantages are not great enough and the results are not consistent enough to warrant a positive opinion on this method.

The sodium acetate treatment is the only method found which has shown positive and fairly consistent stabilizing results.

It must be borne in mind that the only problem is to secure a stable tincture. We have had no difficulty for thirty years in making tinctures which represent the drug satisfactorily, when first made, but these tinctures are instable.

The amount of anhydrous sodium acetate needed is 60 Gm. in 1000 cc. This is equivalent to a little more than 99 Gm. of the U. S. P. sodium acetate containing three molecules of water.

That will equal about 1.5 grains of the official salt in 15 minims of Tincture of Digitalis. The average dose of sodium acetate is given as 25 grains. It has a diuretic action, this being in harmony with digitalis action. It is not a very active or toxic salt. It does not seem likely that it will interfere with or modify the action of the tincture in any quantity which may be given.

The tests on toxicity, which are reported above, were made with the anhydrous salt.

The following formula is offered as a definite subject for consideration:

TINCTURA DIGITALIS.

Tincture of Digitalis.

Tr. Digit.	Digitalis tinctura P. I.
Digitalis, in fine powder	100 Gm.
Glacial Acetic Acid	10 cc.
Anhydrous Sodium Acetate	60 Gm.
To make about	1000 cc.

Pack the digitalis firmly in a cylindrical glass percolator provided with a stopcock and arranged with a cover and receptacle suitable for volatile liquids, and percolate slowly with purified petroleum benzin until a few drops of the last percolate evaporated from paper leave no greasy stain. Reject the benzin percolate.

Remove the drug from the percolator and expose it to air until dry and the odor of benzin is no longer noticeable. Extract this defatted drug by percolation, using a mixture of 4 volumes of alcohol and 1 volume of water as the menstruum, after macerating three days and then percolating slowly. Collect 920 cc. of percolate, add to this the glacial acetic acid and then dissolve the anhydrous sodium acetate in the mixture. Assay a portion of this liquid and dilute the remainder with sufficient of a solution composed of 80 cc. of alcohol, 20 cc. of water, 1 cc. of glacial acetic acid and 6 Gm. of anhydrous sodium acetate to conform to the above biological standard.

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THE GERMICIDAL ACTION OF 2-CHLORO-4-*n*-ALKYLPHENOLS.*

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During the last few years a considerable number of new phenolic germicides have been introduced as therapeutic agents—compounds in which the antiseptic value of the phenolic nucleus has been augmented by the introduction of nuclear halogen or alkyl groups or by both types of substituents; for example, hexylresorcinol, *n*-amyl-*m*-cresol, chlorothymol and chlorocarvacrol.

It seemed to us that a very effective manner in which the germicidal power of phenol itself could be increased would be through the introduction of halogen and a long, straight side chain. Consequently, a homologous series of 2-chloro-4-*n*-

TABLE I.—2-CHLORO-4-*n*-ALKYLPHENOLS AND CORRESPONDING α-NAPHTHOATES.

Alkyl Group	B. P., ° C.	Phenols, Formulas.	Analyses, % Cl. Calc'd.	Found.	α-Naphthoates,[b] M. P., ° C.	Analyses, % Cl. Calc'd.	Found.
Methyl[a]	197–198, 738 mm.	C_7H_7OCl	24.88	25.14	108–110	11.95	12.03
Ethyl	216–217, 742 mm.	C_8H_9OCl	22.64	22.51	70–72	11.41	11.44
Propyl	226–227, 741 mm.	$C_9H_{11}OCl$	20.78	20.34	71–73	10.92	10.78
Butyl	243–244, 735 mm.	$C_{10}H_{13}OCl$	19.21	18.87	44–46	10.47	10.28
Amyl	259–260, 740 mm.	$C_{11}H_{15}OCl$	17.85	17.38	63–65	10.05	10.03
Hexyl	275–276, 740 mm.	$C_{12}H_{17}OCl$	16.67	16.00	43–45	9.67	9.71
Heptyl	290–291, 738 mm.	$C_{13}H_{19}OCl$	15.64	15.53	45–47	9.31	9.12

[a] This compound was first prepared by Schall and Dralle (*Ber.*, 17 (1884), 2528, and then by Zincke (*Ann.*, 328 (1903), 277). The last-mentioned investigator recorded the boiling point as 194–196°. [b] The α-naphthoate of *p*-cresol melts at 61–63°, the diphenyl-*p*-carboxylate at 122–124°. The benzoate of 2-chloro-4-methylphenol melts at 67–68°, the *p*-nitrobenzoate at 88–90°, the diphenyl-*p*-carboxylate at 111–113°. The benzoate of 2-chloro-4-ethylphenol melts at 44–46°.

All of the naphthoates listed in the above table were recrystallized from absolute alcohol; other esters which were found to be too soluble in alcohol were recrystallized from petroleum ether (30–60°). In a few instances the crude naphthoates were somewhat oily, hence they were cooled with ice and thoroughly triturated several times with small amounts of absolute alcohol prior to recrystallization.

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[1] This paper represents the first part of a dissertation to be submitted to the Graduate School by Mr. Stockhaus in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

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