DETERIORATION AND STABILIZATION OF ACONITE PREPARATIONS. PART I.[1,2]

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INTRODUCTION.

The reports of early investigators on the value of aconite as a therapeutic agent were more often conflicting than otherwise. It is now believed that this was due primarily to the fact that the drug and its preparations decreased rapidly in potency due to the instability of aconitine, which is the physiologically active principle. The fact that aconitine is not stable has been known for some time, but it was not until comparatively recent times that attempts to measure accurately its rate of deterioration have been made, and likewise to stabilize the preparations of aconite and aconitine.

In their studies of the deterioration of aconite preparations Swanson (1), Squibb Research Laboratories (2) and Swanson and Hargreaves (3), found that these preparations deteriorated rapidly within a year. It was shown that deterioration is due to the hydrolysis of aconitine into benzaconine and aconine, and that deterioration is directly dependent upon the hydrogen-ion concentration of aconite preparations.

With the object in view of determining the rate of deterioration of aconite preparations (this paper covers only the tincture) and of determining which of the stabilizers in use are the most efficient, the following experimental studies were made. Incidentally, the accuracy of the U. S. P. X method of assay was also studied.

EXPERIMENTAL.

Preparation of Samples.—Dried tubers of *Aconitum napellus* were subjected to examination by a botanist and found to be as represented with respect to identity. The drug was reduced to a No. 40 powder by grinding, and two tinctures of aconite were prepared. Tincture No. 1 was prepared in strict accordance with the U. S. P. X procedure for Tincture of Aconite (7). Tincture No. 2 was prepared by the same method, but with the addition of 2 per cent acetic acid to the 70 per cent alcoholic menstruum, a procedure recommended as being the most desirable to insure stability.

The tincture was selected as the preparation for this study as it is the preparation of aconite most frequently used, and because it is typical of the other liquid preparations of aconite in so far as lack of stability is concerned.

Tincture No. 1.—120-cc. portions of this tincture were measured off by a burette into eleven amber-colored bottles of 125 cc. capacity. Before filling, the bottles were immersed for 24 hours in water acidulated with 2 per cent hydrochloric acid, to neutralize any possible alkalinity of the glass, then thoroughly rinsed with water.

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[1] From the laboratories of A. G. Du Mez, Professor of Pharmacy, and M. R. Thompson, Professor of Pharmacology, School of Pharmacy of the 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, 1933.

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

Each portion, except one, which was used as a control, was acidified with a definite amount of acid as shown below, and the p_H values determined within a day or two.

TABLE I.—p_H VALUES OF PORTIONS.

Portion.	Per Cent Acid Added.	p_H Value.
1	None	5.7
2	0.1 hydrochloric acid	3.8
3	0.2 hydrochloric acid	2.4
4	0.3 hydrochloric acid	2.3
5	0.4 hydrochloric acid	1.9
6	2.0 acetic acid	4.6
7	2.5 acetic acid	4.5
8	3.0 acetic acid	4.45
9	3.5 acetic acid	4.4
10	4.0 acetic acid	4.3
11	2.0 hypophosphorous acid	1.15

Acetic and hydrochloric acids were used for acidifying as they had been used for this purpose by other investigators.

Hypophosphorous acid was used because of its reducing properties and because it was thought that deterioration might be due, in part at least, to oxidation of the alkaloids. The p_H value obtained for this portion was so low (p_H 1.15) that it appeared as though the amount of hypophosphorous acid added (2 per cent) was more than sufficient to give the value (approximately p_H 2.5–3.0), reported by other investigators as being the most desirable. To determine if this was actually the case, 25 cc. of non-acidified tincture were accurately measured off and adjusted for p_H value by adding successive small amounts of acid measured from a burette. The following results were obtained:

TABLE II.—ADJUSTMENT OF p_H VALUE WITH HYPOPHOSPHOROUS ACID.

Cc. of Hypophosphorous Acid Added.	p_H Value.
0.1	3.0
0.2	2.7
0.3	2.6
0.4	2.0

Tincture No. 2.—One liter of tincture was prepared using a 70 per cent alcoholic menstruum acidulated with 2 per cent acetic acid. The tincture was not divided into portions as in the case of Tincture No. 1, but was preserved in one bulk for observation. The p_H value of this tincture was found to be 4.80.

The addition of the same percentage of an acid will give variable p_H values in preparations made from different lots of drugs, due to the variable amounts of alkaloids and inert extractive matter present. Therefore, it is inadvisable to specify what percentage of acid should be added to the preparation for stabilizing purposes. The p_H value that will give the greatest protection to the preparation should be stated instead.

The results of this study show that the use of acetic acid, or even glacial acetic acid, as stabilizers, is impractical because of the comparatively large quantity which must be added to bring the p_H value down to the desired level. The results presented in the following table demonstrate this clearly:

TABLE III.

Hydrochloric Acid. Amount of Acid Added to 30 Cc. of Tincture.	p_H Value.	Acetic Acid (36%). Amount of Acid Added to 25 Cc. of Tincture.	p_H Value.	Acetic Acid (Glacial). Amount of Acid Added to 30 Cc. of Tincture.	p_H Value.
None	5.48	None	4.8	None	4.77
0.1 cc.	2.97	3.8 cc.	3.7	0.5 cc.	4.33
0.2 cc.	1.74	6.2 cc.	3.4	2.5 cc.	3.85
		7.7 cc.	3.3	4.5 cc.	3.47
		10.0 cc.	3.1	7.0 cc.	3.17
		11.7 cc.	3.0	9.0 cc.	2.98
		13.6 cc.	2.8	11.0 cc.	2.82
		17.5 cc.	2.7	14.0 cc.	2.61
		23.4 cc.	2.5	16.0 cc.	2.53
				17.0 cc.	2.43
Percentage of acid added to obtain p_H value of 1.74 = 0.66.		Percentage of acid added to obtain p_H value of 2.50 = 93.60.		Percentage of acid added to obtain p_H value of 2.43 = 56.66.	

Assay of Samples.—The method used for the assay of Tinctures Nos. 1 and 2 is the one described in the U. S. P. X (7) under Tincture of Aconite. The samples prepared were assayed at intervals of approximately three months.

The portions to be assayed were diluted so that the dose to be administered to the animal would come within a convenient injection range (0.4–1 cc.). Warm tap water, chlorine free, was used for making the dilutions.

TABLE IV.—RATE OF DETERIORATION OF TINCTURE NO. 1.

			Date of Assay.							
Portion.	Acid Used.	p_H.	10–29–31. Per Cent Strength.	1–29–32. Per Cent Strength.	5–2–32. Per Cent Strength.	8–2–32. Per Cent Strength.	11–28–32. Per Cent Strength.	3–12–33. Per Cent Strength.	3–8–34. Per Cent Strength.	p_H.
1	None	5.7	114	66	Below 50	...	...	...	...	5.56
2	HCl	3.8	...	89	80	...	...	Below 50	...	4.40
3	HCl	2.4	...	100	...	89	72	...	...	2.89
4	HCl	2.3	...	114	...	114	...	114	114	2.85
5	HCl	1.9	...	114	...	114	114	100	...	2.36
6	CH_3COOH	4.6	...	100	66	Below 50	...	...	...	4.72
7	CH_3COOH	4.5	...	100	...	...	Below 50	...	...	4.63
8	CH_3COOH	4.45	...	89	80	...	...	...	...	4.56
9	CH_3COOH	4.4	...	100	...	100	Below 50	...	...	4.57
10	CH_3COOH	4.3	...	114	114	114	Destroyed	...	...	...
11	H_3PO_2	1.15	...	114	114	114	80	72	...	1.46

The U. S. P. X (7) gives the minimum lethal dose for Tincture of Aconite as 0.00035 cc. to 0.00045 cc. per Gm. weight of guinea pig. Therefore, a tincture, the minimum lethal dose of which is found to be 0.00040 cc. per Gm. weight of animal, would be considered to be 100 per cent with respect to the U. S. P. standard.

Immediately after preparation, Tinctures Nos. 1 and 2 were assayed and found to have a potency of 114 per cent.

The acid used to acidify the portions of Tincture No. 1 apparently had no effect on the animals, as the minimum lethal dose was found to be the same in all cases, as shown in the table which follows. This table also shows the rate of deterioration as revealed by assays made at intervals, in all but one instance, of approximately three months.

The stabilizing effect of the addition of acid is clearly shown by the foregoing results. Tinctures prepared without the addition of acid deteriorate rapidly. Hydrochloric acid is shown to be the best stabilizer of the acids employed. This is demonstrated particularly well by the assay results for "portion 4" of Tincture No. 1, which had an initial p_H value of 2.30. In this case, hydrochloric acid appears to have afforded complete protection over a period of twenty-nine months. In other portions acidified with hydrochloric acid the protection was not so striking, deterioration increasing with an increase in p_H value.

TABLE V.—RATE OF DETERIORATION OF TINCTURE NO. 2.

	Date of Assay.				
	10–29–31. Per Cent Strength.	1–29–32. Per Cent Strength.	8–2–32. Per Cent Strength.	3–12–33. Per Cent Strength.	
p_H.					p_H.
4.8	114	100	89	72	4.94

Acetic acid proved less efficient than hydrochloric acid. It retarded deterioration to a moderate extent when added in sufficient amount. When acetic acid was added to the menstruum instead of the percolate, as shown by Tincture No. 2, deterioration also took place, although not to the same extent as when the same percentage of acid had been added to the percolate as is the case in "portion 6" of Tincture No. 1.

Hypophosphorous acid, likewise, afforded only moderate protection.

Based on the foregoing results it may, therefore, be said that hydrochloric acid is preferable to acetic acid as a stabilizer for tincture of aconite for the following reasons:

I. It possesses greater ionizing power than acetic acid.

II. Having better ionizing properties, much less hydrochloric acid is necessary for adjusting the preparation to the desired p_H value than when acetic acid is used.

Hydrochloric acid is preferable to hypophosphorous acid as a stabilizer for tincture of aconite because it possesses greater ionizing power and is more economical.

Approximately four months after Tinctures Nos. 1 and 2 had been prepared, two other tinctures were prepared from the same lot of drug. These two tinctures were numbered 3 and 4, respectively.

Tincture No. 3.—This tincture was prepared from the same lot of powdered aconite tubers used in preparing Tinctures Nos. 1 and 2, the only difference being that in this case the drug had been in the powdered condition for four months, whereas in the preceding cases the drug was ground just before the preparation of the tinctures was begun.

Tincture No. 4.—This tincture was prepared from the drug which had been *kept whole*, and powdered at the end of the four-month period.

The two tinctures, prepared as described, were assayed for potency with the following results:

TABLE VI.—RATE OF DETERIORATION OF DRUG.

Tincture.	Strength in Per Cent.	Assay Date.
No. 3	57	2–15–32
No. 4	73	2–15–32

In the case of Tincture No. 3 where the aconite had been kept over a period of four months in the powdered condition before being made into tincture, the drug shows a loss of 57 per cent, or about half of its strength compared with the results obtained in the initial assays of Tinctures Nos. 1 and 2.

In the case of Tincture No. 4 where the aconite had been kept over this period of time in the *whole* condition before being made into tincture, the drug shows a loss of 41 per cent compared to the initial strengths of Tinctures Nos. 1 and 2.

It appears, therefore, that aconite should not be kept in the powdered condition for any lengthy period of time, but that it should be stored in the whole condition and powdered just previous to making up into tincture or other preparation.

Aconitine.—Swanson and Walters (8) assert that the deterioration of aconite preparations cannot be definitely determined until a more satisfactory method of assay has been developed. Swanson (1) feels that the lethal dose method would be satisfactory for testing the therapeutic efficacy of aconite, providing the total alkaloids were determined in terms of a standard aconitine, and a standard method of technique was employed.

With the intention, therefore, of studying the seasonal variation of guinea pigs (their susceptibility to a stable standard substance at different intervals during the year), and of the use of aconitine as the standard, the following work was undertaken:

Crystalline aconitine (Aconitine Potent, Merck) was recrystallized repeatedly from hot alcohol until its toxicity to guinea pigs became constant. This aconitine, used as a standard throughout the work, was recrystallized six times. The crystals were then dried thoroughly, and sealed *in vacuo* in portions of 20–30 mg. to the ampul.

"Aconitine values," using this pure crystalline aconitine, were determined at each interval of assay of the Tinctures Nos. 1 and 2 (approximately three-month intervals). A 1:50,000 dilution of hydro-alcoholic solvent (about 25 per cent alcohol) constituted the working solution for injection.

Healthy, normal guinea pigs were used for the determination of the aconitine values. These animals had been obtained from one source only, and had been acclimated to laboratory surroundings before being used for assay purposes.

The U. S. P. X method of assay as directed under Tincture of Aconite (7) was followed in the determination of the minimum lethal dose as in the case of the tinctures assayed.

TABLE VII.—ACONITINE VALUES. GM. ACONITINE PER GM. WEIGHT OF GUINEA PIG.

10–29–31.	1–29–32.	5–2–32.	8–2–32.	11–28–32.	3–15–33.
0.000000050	0.000000055	0.000000050	0.000000055*	0.000000055	0.000000050

* In obtaining the "aconitine value" on date of "8–2–32," the weight of one of the guinea pigs used was 465 Gm., which is considerably beyond the U. S. P. range. This procedure was made necessary, however, due to a shortage of guinea pigs at that time. Apparently, however, this did not affect the results to any noticeable extent.

The foregoing aconitine values show that no appreciable change in susceptibility of the guinea pigs to the aconitine took place in 15 months. This is explained by the fact that the guinea pigs used were in an absolutely healthy, normal condition and had been obtained from one source only. This, probably, would not be the

case if the condition of the guinea pigs was poor, if the animals had not been acclimated to laboratory surroundings and if they had been obtained from various sources. This matter is receiving further consideration.

CONCLUSIONS.

1. The use of hydrochloric acid as a stabilizer for tincture of aconite is superior to acetic acid, provided the preparation is adjusted to the correct hydrogen-ion concentration.

2. The use of acetic acid as a stabilizer is impractical because of the large amount required to adjust the preparation to the desirable hydrogen-ion concentration.

3. Aconite in the form of the whole crude drug deteriorates rapidly and the powdered drug deteriorates even more rapidly.

4. Tincture of Aconite U. S. P. X, because of its poor keeping qualities, should be adjusted with hydrochloric acid to a hydrogen-ion concentration sufficient to preserve it (approximately, p_H 2.3–3.0).

5. The resistance of guinea pigs to aconite, or aconitine, is consistent provided the weight range specified by the U. S. P. X is strictly adhered to, and further provided that these guinea pigs are in an absolutely healthy, normal condition.

6. The advisability of the use of aconitine as a bioassay standard will receive more extensive consideration in a later report.

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Henry Ford says he has had hunches, but what others call hunches he calls memories of things learned in past lives; "study the past," he says "and you will see that the congresses and the crowds were always arguing irrelevant and unimportant issues while the real revolution was going on quietly in the laboratory."