PAPER NO. 2. THE STANDARDIZATION AND STABILIZATION OF ACONITE PREPARATIONS.*

BY EDWARD E. SWANSON.

I. Review of previous data.
II. Reassay of aconite preparations.
III. The alkaloids of aconitum napellus.
IV. The hydrogen ion concentration factor.
V. Conclusions.

I. REVIEW OF PREVIOUS DATA.

In a previous article (1), it was shown that the chemical method of the U. S. Pharmacopœia IX revision is not reliable for standardizing aconite preparations. This method assays the total ether-soluble alkaloids, which are similar in chemical properties toward solvents and precipitants, but not similar in toxicity and pharmacological action. The biochemical method, which has been found to be more accurate, determines the total amount of toxic alkaloids in terms of a standard aconitine, which is regarded pharmacologically and therapeutically as the important alkaloid. However, its efficiency not only depends upon the *aconitine value*, but also upon a standard method of *technic*. The accuracy of the method depends upon a standard weight of guinea-pigs, the seasonal variation of guinea-pigs, the acclimation of guinea-pigs to laboratory surroundings, the standardization of various lots of guinea-pigs, the use of starved or non-starved guinea-pigs, the dilution of the preparations to be tested, and finally the amount to be injected. These factors, or problems, are now under investigation and will be reported in another article.

A comparison of the chemical and biochemical methods on a number of aconite drugs, tinctures and fluidextracts not only shows that the chemical method is unreliable, but also that the tinctures and fluidextracts deteriorate rapidly within a year; and furthermore that this loss in activity can be partially or totally prevented by the addition of acetic acid, or hydrochloric acid, in the finished percolate or menstruum. The preparations were assayed during the year and at the end of the year. The writer has reported the results of one, two, and two and one-half years' aging, and now reports the results of three or more years' aging.

To briefly summarize the previous reported data:

Tinctures 1, 4 and 7, prepared according to the U. S. P. method, deteriorated rapidly in one year's time as follows:

Preparation.	Date.	Per cent. Bio-assay.	Date retested.	Per cent. Bio-assay.
Tincture 1	11/20/20	181	11/28/21	13
Tincture 4	11/20/20	333	11/28/21	43.6
Tincture 7	6/12/21	100	11/28/21	12

Tinctures 2, 5 and 8, prepared according to the U. S. P. method with 2% acetic acid, or 0.1% hydrochloric acid added to the finished percolate, assayed as follows:

Preparation.	Date.	Per cent. Bio-assay.	Date retested.	Per cent. Bio-assay.
Tincture 2	11/20/20	181	12/25/22	93
Tincture 5	11/20/20	333	12/25/22	266
Tincture 8	6/12/21	128	12/25/22	114

* Scientific Section, A. Ph. A., Buffalo meeting, 1924.

The tinctures 3 and 6, prepared with a 2% acetic acid 70% alcoholic menstruum, showed even less deterioration, if any, as follows:

Preparation.	Date.	Per cent. Bio-assay.	Date retested.	Per cent. Bio-assay.
Tincture 3	11/20/20	166	12/25/22	166
Tincture 6	11/20/20	335	12/25/22	333

II. REASSAY OF THE SAME PREPARATIONS.

Preparation.	Date.	Per cent. Bio-assay.	Date retested.	Per cent. Bio-assay.
Tincture 2	11/20/20	181	11/ 2/23	94
Tincture 5	11/20/20	333	11/20/23	228
Tincture 8	6/12/21	128	11/ 2/23	94
Tincture 3	11/20/20	166	11/15/23	160
			6/18/24	150
Tincture 6	12/20/20	335	11/29/23	320
			6/10/24	310

The above data shows that tinctures 2, 5 and 8, prepared with 70% alcohol and acid added to the finished percolate, have either retained or are gradually deteriorating, while the tinctures 3 and 6, prepared with an acid alcohol menstruum, seem to have retained their activity.

III. ALKALOIDS OF ACONITUM NAPELLUS.

The alkaloidal content of *Aconitum Napellus* was first studied by Geiger and Hesse (2) and Von Planta. (3) However, Grove (4) was the first to obtain a crystalline form. Wright (5) and his pupils prepared several salts and studied their properties. Dunstan (6) and his collaborators, and Freund (7) and his pupils, found that the principal alkaloids of *Aconitum Napellus* are (1) aconitine the principal alkaloid, (2) benzoyl aconine and (3) aconine. The alkaloid aconitine contains four methoxyl groups and a methyl group linked to a nitrogen atom. By heating aconitine in an aqueous solution under pressure it undergoes hydrolysis, forming first acetic acid and benzoyl aconine, which occurs in the aconite root, and eventually benzoic acid and aconine, which also occurs as such in the plant. These reactions may be represented thus:

(1) $(HO)_3(CH_3O)_4C_{20}H_{21}NMe(O.CO.C_6H_5)(O.CO.CH_3) + HOH = (HO)_3(CH_3O)_4C_{20}H_{21}NMe(O.CO.C_6H_5)(H) + CH_3COOH$

Aconitine — Benzoyl aconine — Acetic acid

(2) $(HO)_3(CH_3O)_4C_{20}H_{21}NMe(O.CO.C_6H_5)(H) + HOH = (HO)_3(CH_3O)_4C_{20}H_{21}NMe(H)(H) + C_6H_5COOH$

Benzoyl aconine — Aconine — Benzoic acid

The aconite alkaloids undergo decomposition, especially hydrolysis, with remarkable ease. Dunstan and Ince (8) found that the pure crystalline aconitine, when heated to its melting point, was completely decomposed, but was not affected by exposure to a temperature of 100–120° for one hour. Wright and Luff (5) and Dunstan and Carr (9) have shown that aconitine is completely decomposed into benzoyl aconine and aconine by prolonged heating with water in a closed tube at

100° C. Also, that if the alkaloid is dissolved in an alkaline water solution in closed tubes the decomposition is even more rapid, forming acetic acid, benzoic acid and aconine; and finally that the alkaloids dissolved in an alkaline alcoholic solution decompose rapidly. Dunstan and Carr(10) found that the hydrolysis of aconitine may be affected by heating with H_2O_2, forming acetic acid and benzoyl aconine, and completing the reaction to benzoic acid and aconine by heating with aqueous alkali or strong acids. Therefore, partial hydrolysis forms benzoyl aconine, and complete hydrolysis forms aconine. Swanson and Walters(1) have shown that aconitine crystals dissolved in 70% alcohol decomposes very rapidly at room temperature.

Considering the above review of the literature, the deterioration of aconite preparations may be due to hydrolysis or a chemical change of aconitine into benzoyl aconine and aconine. In order to study the problem more closely the writer has prepared solutions of the alkaloids aconitine, benzoyl aconine and aconine (obtained through the courtesy of Dr. A. R. L. Dohme and Dr. H. Engelhardt, Sharp & Dohme Co., Baltimore, Md.). Diacetyl aconitine, which is regarded as one of the important derivatives of aconitine, was prepared according to Dunstan's method by adding acetic anhydride to aconitine crystals in molecular proportions at room temperature, and allowing to stand for 10 hours. Each alkaloid was dissolved in 70% alcohol, and 70% alcohol plus 2% acetic acid, which assayed as follows:

Alkaloid.	Date tested.	Solution.	L.D. per Gm. wt.
Aconitine	11/ 5/23	70% alcohol	0.000000060
Aconitine	6/13/24	2% acetic acid	0.000000055
Aconitine	11/15/23	70% alcohol	0.00000010
Aconitine	6/20/24		0.0000015 to 0.0000020
Diacetyl aconitine	11/29/23	70% alcohol	0.000000575
Diacetyl aconitine	6/18/24	2% acetic acid	0.000000575
Diacetyl aconitine	11/29/23	70% alcohol	0.00000060
Diacetyl aconitine	6/18/24		0.00000065
Benzoyl aconine	11/26/23	70% alcohol	0.000030
Benzoyl aconine	7/ 9/24	2% acetic acid	0.000035
Benzoyl aconine	11/26/23	70% alcohol	0.00015
Benzoyl aconine	7/ 9/24		No deaths at 0.00015 (Insufficient material)
Aconine	12/20/23	70% alcohol	0.00025 to 0.00030
Aconine	7/ 9/24	2% acetic acid	0.00030
Aconine	12/20/23	70% alcohol	0.00075 to 0.0010 (Insufficient material)

The above data shows that the pure alkaloids dissolved in 70% alcohol decompose, and this decomposition, or hydrolysis, similar to the deterioration of the tincture, is prevented by the presence of an acid. In an acid alcoholic solution the alkaloids assay as follows:

Aconitine	= 0.000000060 Gm. per Gm. wt.
Diacetyl aconine	= 0.00000060 Gm. per Gm. wt.
Benzoyl aconine	= 0.000030 Gm. per Gm. wt.
Aconine	= 0.00030 Gm. per Gm. wt.

Therefore, aconitine is about 10 times as toxic as diacetyl aconitine, 500 times as toxic as benzoyl aconine, and 5000 times as toxic as aconine.

IV. HYDROGEN ION CONCENTRATION FACTOR.

Wright and Luff(5) state that aconite alkaloids in an alkaline alcoholic solution hydrolyze with remarkable ease. The U. S. P. tinctures and fluidextracts of aconite do not give an alkaline reaction toward litmus when freshly made or when aged, however, this is probably prevented by the alcohol. The alkaloids of aconite are probably in combination with weak acids and decompose readily into the free base. Macht and Fisher(11) found that alkaloids containing unoxidized benzyl nuclei exhibit particularly great toxicity. Crane(12) determined the toxicity of several unrelated alkaloidal substances for paramecium, and observed that in general the toxicity of alkaloids having a large dissociation constant tends to be much influenced by the hydrogen ion concentration of the medium. Bills and Macht(13) found that opium alkaloids at various hydrogen ion concentrations differ in toxicity to paramecium. Therefore, in view of these findings, the toxicity, deterioration and stabilization of aconite preparations and their alkaloids may be a question of hydrogen ion concentration. The above aconite preparations have the following p_H value.

Preparation.	P_H value.	
70% alcohol (control)	5.0	Rapid deterioration
Tincture 1 U. S. P.	5.6	
Tincture 4 U. S. P.	5.6	
Tincture 7 U. S. P.	5.65	
Tincture 2 (acetic acid in finished percolate)	4.2	Partial deterioration
Tincture 5 (acetic acid in finished percolate)	4.65	
Tincture 8 (HCl in finished percolate)	2.3	
Tincture 3 (acid menstruum)	5.00	No deterioration
Tincture 6 (acid menstruum)	5.00	

The above data does not show a distinct variation in hydrogen ion concentration. However, the preparations containing an acid give a higher p_H value than the U. S. P. preparations. The difference is due to the poor dissociating property of acetic acid compared with that of hydrochloric acid. Since the presence of an acid, or the hydrogen ion concentration factor, seems to prevent hydrolysis of the aconite alkaloids, or prevents the deterioration of the tinctures and fluidextracts, the problem of comparing the two methods on a series of preparations with various hydrogen ion concentrations in regard to stability and deterioration is still to be investigated and will be reported later.

V. CONCLUSIONS.

The experiments reported on deterioration show that the deterioration of the tinctures of aconite can be prevented by the addition of an acid to the finished percolate or menstruum.

The experiments on the decomposition of the pure alkaloids of aconite show that the alkaloids do not decompose or hydrolyze in an acid alcoholic solution. That the deterioration of the tinctures and fluidextracts is probably due to decomposition or hydrolysis of the alkaloids, and may be a hydrogen ion concentration factor.

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COD-LIVER OIL AND ITS BY-PRODUCTS.*

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Previous to the general adoption of the steam process, medicinal cod-liver oil was made by the direct fire method. At a still earlier period cod-liver oil was made by the rotting process. All three of these processes have desirable features. The steam process produces the best oil, but it requires considerable equipment and skilled labor. The direct fire process can be used when steam is not available, and experienced operators can produce very good oil by this method especially if water is used in the liver kettle. The rotting process requires practically no equipment or expense for labor. As a result all the processes are in use at the present time and a variety of oils are obtained, which range in appearance from an attractive straw yellow, edible cod-liver oil to a ruby red, nauseating, heavy oil known in the trade as "cod oil."

The uses to which the oils made by these processes are put are as diversified as the nature and the quality of the oils. They vary from the therapeutic use of highly potent medicinal oil as a source of the fat-soluble vitamins to the use of "cod oil" for industrial purposes.

Since the nature, value, and use of cod-liver oil depends to a large measure on the method of manufacture, it is of interest to sketch briefly some of the general conditions surrounding the manufacture of cod-liver oil and its by-products.

To make the highest quality cod-liver oil the livers from healthy fish should be removed as soon as possible after the fish are caught. They should be rendered very promptly, preferably by the steam method. As soon as the livers are thoroughly cooked, the oil will rise to the surface of the kettle and it should be skimmed off at once. As rapidly as possible it should be freed of water and all particles of liver tissue. The oil is then chilled and separated into non-freezing medicinal cod-liver oil and cod-liver stearin. Unless the oil is to be used at once it should be promptly bottled and sealed to protect it from the oxidizing action of the air.

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