

2. Reduction of molecular mixtures of the different arsenic acids with sodium hydrosulphite is not a general method for the production of unsymmetrical derivatives.

3. The loss of one of the essential anchoring groups, according to Ehrlich, from arsphenamine does not impair its therapeutic efficiency as far as these laboratory tests show.

In conclusion we wish to thank Dr. Frederick W. Heyl at whose suggestion this work was carried out.

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ALOIN.

BY H. ENGELHARDT AND H. H. CROSBIE.

Aloin is defined in the United States Pharmacopœia as a pentoside or a mixture of pentosides, *i. e.*, compounds which resemble the glucosides and differ from them only in that one of the components is not a carbohydrate proper, but a five-atomic alcohol. Until about 20 years ago aloin was classed among the bitter principles, until Léger found that on prolonged hydrolysis with alcoholic hydrochloric acid it is split up into 1.8 dioxi-3 methylenoxianthraquinone and *d*-arabinose.

While introducing refinements in the process of manufacture, with the idea of greater purity, some difficulty was encountered with the solubility of aloin in water, which the U. S. P. states is 1 : 120. It was considered possible that in the manufacturing process of aloin under certain physical conditions (concentration, temperature and rate of crystallization) a product was obtained, which contained a larger amount of other less soluble pentosides. It was found that when shaking aloin with water in the proportion of 1 : 120, an apparently large amount of silky needles remained undissolved which made the solubility of the aloin not respond to the official requirement, *i. e.*, that not more than 1.5 per cent. of the aloin should be insoluble in the prescribed amount of water. The present U. S. P. Revision Committee proposed that the amount of water-insoluble matter be reduced to one per cent. and it was, therefore, interesting to find out whether or not aloin made by different reputable manufacturers would come up to this requirement. It was found that only one of these aloins out of four contained less than one per cent. of water-insoluble matter and this specimen had a pronounced odor of aloes which suggested that the mother-liquid had only insufficiently been removed. It was also found that a specially pure sample, which showed insufficient solubility, could be made to conform to U. S. P. requirements by the addition of 5% of powdered aloes, apparently a serious reflection on the test. And experiments showed that the solubility of aloin can be somewhat increased by not completely eliminating the mother liquid, but we believe that by doing so an aloin is obtained which is not completely devoid of the griping principles of the drug. To be frank, the insufficient solubility of the aloin was not due to the presence of other compounds (pentosides?) but to the temperature at which the solubility test had been carried out, because an aloin which when shaken with water in a

proportion 1 : 120 at ordinary laboratory temperature, gave 2 per cent. insoluble matter, left, when shaken at exactly 25° C. for two hours, only 0.7 per cent. of insoluble matter. When the shaking was done at 16° C., as much as 7.0 per cent. of the aloin remained undissolved. The solubility of aloin is therefore so dependent on the temperature that special mention should be made of the necessity of maintaining standard temperature.

It was further found that crystals over 100 μ in length have a solubility less than that shown by crystals of average length of 10 μ . Yet it is necessary to have a certain per cent. of this larger size in order to be able to eliminate last traces of mother liquor.

Confirmatory experiments were tried to test the theory that aloin was not a mixture, and these experiments may be of interest.

Having previously found that aloin when treated with an insufficient amount of acetone leaves a residue of a decidedly grey color, it had been assumed that separation could thus be effected by means of this solvent. On going into the matter more thoroughly it was found that when aloin was divided into 3 portions (by means of shaking with successive insufficient quantities of acetone and crystallizing these fractions) all three fractions had the same solubilities and melting points when *repurified by crystallization from water*. There is a breaking down of the aloin going on, the cleavage product being of a purple color which with the yellow of the aloin gave the gray color mentioned.

Fractional crystallization of aloin from water gave the same results.

The details of the experiments were as follows: An aloin which contained 6.5 per cent. of water of crystallization, was soluble at *ordinary* laboratory temperature to an extent of 97.6% and melted at 129°, was chosen for this purpose, 3 lbs. of this aloin was shaken for two hours with 15 pints of acetone. The mixture was then filtered under reduced pressure when a *greyish yellow* substance remained. The filtrate was concentrated in vacuo and the crystals which separated were recrystallized from water. They were of a beautiful golden yellow color, contained 7.65% of water of crystallization and melted at 134°. The greyish yellow substance, not dissolved in the first portion of acetone, was then shaken for two hours with 20 pints of acetone and the mixture was filtered again. A greyish purple substance remained undissolved. The filtrate was treated as above, *i. e.*, evaporated in vacuo and an aloin, also golden yellow in color, which contained 7.9 per cent. of water of crystallization and which melted at 138° was obtained.

The substance undissolved in acetone was shaken with 40 pints of the solvent, when practically all went into solution leaving only a small dark purple residue, which was not further examined. This third filtrate, which appeared more contaminated with cleavage products than the former filtrates, was treated differently. When concentrated and allowed to cool the cleavage products separated as an amorphous solid, which was quickly removed by filtration, before crystallization of the aloin had started. This amorphous solid was readily soluble in water, yielding a deep purple solution.

The aloin which crystallized from the filtrate was yellow and on recrystallizing from water had a brilliant yellow color, contained 6.5 per cent. of water of crystallization and melted at 138°.

These three fractions of aloin were almost completely soluble in water when

tested by the official method at ordinary temperature. This evidence, *i. e.*, almost identical melting points, solubilities and their water of crystallization confirms the theory that aloin is *one* chemical substance (apart from small amounts of unavoidable cleavage products). In regard to the melting points it may be said that these were not entirely sharp. The different specimens of aloin sintered at about 110° , but then melted rather sharply at the above cited temperatures. That the original aloin was not quite as pure as the three fractions is shown by the melting point, 129° , but one has to consider that even infinitesimal quantities of cleavage products, which are unavoidable to be produced in the manufacturing of products of this kind, are liable to depress the melting point considerably.

A further experiment was very interesting. One pound of aloin was shaken for two hours with 100 lbs. of water and the temperature kept low enough to give an undissolved residue, representing 10 per cent. of the original aloin. This residue was dried and under the microscope showed needle crystals, the larger being 110μ long. On recrystallizing these needles from water under conditions favorable for the development of small crystals, needle crystals not exceeding 12μ in length were obtained. The latter showed a much better solubility when submitted to the U. S. P. test than the former, leading to the conclusion that the variations in solubilities found have a physical and not a chemical cause. And that the solubility of aloin is entirely dependent on the temperature at which the dissolving is carried out, as already mentioned, is shown by the following experiments. The original aloin taken in these experiments was shaken with water in the proportion 1 : 120 at 15° , when 7 per cent. remained undissolved. When shaken at 20° , 2.3 per cent., at 22.5° , 1.0 per cent. and at the official temperature 25° , 0.2 per cent. remained undissolved.

On various occasions we have been asked if there were a reliable method for estimating aloin in aloes. Our answer always has been "no." Quite a number of methods have been recommended but by none can the amount of aloin even approximately be determined.

Tschirch suggested, in order to evaluate aloes, to extract aloes with a mixture of methyl alcohol and chloroform, by which only the active substances are dissolved, while the inactive resins remain undissolved. This method cannot be considered as reliable because it cannot be accepted that the amount of crystalline aloin is always the same in the active resins.

Schäfer's method which depends on the extracting of aloes with water, rendering the solution alkaline with ammonia and then precipitating the aloin by means of calcium chloride, etc., gives wholly unreliable results, because they do not agree with the amount of crystalline aloin afterwards obtained. The resulting product appears to be something other than pure aloin.

Having found that aloin gives a highly fluorescent solution with borax solution, Schotenten thought of utilizing this property of aloin for estimating it in aloes. The drug was extracted with water, to the aqueous solution a strong borax solution was added and the liquid was then diluted with water until a just perceptible fluorescence was exhibited, it having previously been found that such a fluorescence is produced by a solution of pure aloin in borax solution in a dilution of 1 : 250,000. From the amount of aloes taken and the dilution, he claimed that the percentage of aloin could easily be calculated. This claim is not justified at all, because the

amorphous substances, which are nearly related to crystalline aloin, give this fluorescence with borax solution also.

The following experiments, which were made quite a number of years ago, may be of interest.

A large quantity of aloes was worked up in the process of making aloin, and the material was tested by this method both before and after the extraction of the crystals.

The test gave a higher figure for aloin content *after* the aloin had been removed than before, and neither figure had any relation to the actual amount found, which was normal.

This again shows that these resins are nearly related to crystalline aloin, a claim which is also corroborated by the fact, that when treated with Caro's acid both the crystalline aloin and these resinous substances yield tri- and tetra-oximethylantraquinones.

The precipitation of aloin as tri-brom-aloin by means of bromine water gives unreliable results also, because the amorphous oximethylantraquinones are precipitated together with the aloin.

The only way we know of to determine the amount of aloin in aloes is to work up about 10 pounds of aloes; but such a process is not very feasible, because while at times under certain conditions the aloin may crystallize out within a few days, under other conditions, especially when a larger amount of amorphous substances than usual is present, the complete crystallization may take weeks.

It is to be regretted that up to the present time no reliable assay process is at our disposal for such a valuable and widely used drug as aloes.

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A SOURCE OF ERROR IN EMPLOYING FUCHSIN-SULPHUROUS ACID SOLUTION AS A TEST FOR FORMALDEHYDE IN ETHYL ALCOHOL.*

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The U. S. P. test for methyl in ethyl alcohol depends upon the oxidation of the methanol by means of potassium permanganate to formaldehyde and the detection of the latter by fuchsin-sulphurous acid in the presence of sulphuric acid.

The fuchsin-sulphurous acid solution is known as Schiff's Reagent and is employed for the detection of aldehydes in general, but taking advantage of the fact that sufficient sulphuric acid prevents development of color by acetaldehyde which is the product formed when ethyl alcohol is oxidized, Denigès suggested that the fuchsin-sulphurous acid solution be added in the presence of sulphuric acid, and in this form the test is official in the U. S. P.

In the final steps of the test there are added to the oxidized material one cc of strong sulphuric acid and five cc of fuchsin-sulphurous acid solution and if a distinct blue or violet color is produced formaldehyde is indicated; as this results from oxidizing methyl alcohol the latter is reported.

* Read before New York State Pharmaceutical Association, Stamford meeting, 1923.