

NOTES ON THE ESTIMATION OF MORPHINE AND ON LLOYD'S REAGENT.

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1. *Attempt to shake out morphine with a mixture of alcohol and chloroform from a saturated solution of potassium carbonate.*

When a saturated solution of potassium carbonate is shaken with alcohol or a mixture of equal volumes of alcohol and chloroform, most of the alcoholic solvent very quickly separates out on the surface of the heavier aqueous layer. This was proved by adding a definite volume of the alcoholic liquid to an equal volume of the saturated solution of potassium carbonate, shaking the mixture vigorously, and reading off the volume of the upper layer after the liquid has separated in two layers. In all cases the volume of the alcoholic layer was only a little less than the volume of the alcoholic liquid originally taken.

Owing to there being no good immiscible solvent for the extraction of morphine from the solution of its salts in water, an attempt was made to saturate such a solution with potassium carbonate, and to use a mixture of equal volumes of alcohol and chloroform as an immiscible solvent. The aqueous liquid, after being shaken once with an equal volume of the alcoholic liquid, using about 40 cc. of each for about 0.1 g. of morphine in the form of salt, gave no test, in acidified solution, with Mayer's or Wagner's reagent, while, on the other hand, the alcoholic liquid was found to contain, besides morphine, small amounts of potassium carbonate, together with small amounts of other substances, coming either from impurities in the carbonate, or from a partial decomposition of morphine by the latter, or from both sources. Even when the potassium carbonate was previously washed with alcohol and dried, the alcohol-chloroform-solution of the morphine contained small amounts of other substances.

It was thought that by washing the residue left, (after distilling off the alcoholic liquid from the morphine), with a saturated solution of the alkaloid, the impurities could be eliminated, so that the morphine could be determined alkalimetrically. For this purpose, definite amounts of morphine were dissolved in acidified water, the solution saturated with potassium carbonate, (either ordinary or previously washed with alcohol), and then shaken with a definite volume of a mixture of equal volumes of alcohol and chloroform. After the complete separation of the liquid into two layers, an aliquot portion of the upper alcoholic layer was drawn off and evaporated to dryness. The residue was washed, with a saturated solution of morphine in water, until the washings gave no test for potassium carbonate with phenolphthalein, and the morphine determined alkalimetrically, using N/25, H₂SO₄ and N/50, KOH. The indicator was methyl-red.

The experiments showed that, in all cases, the amount of morphine found, ex-

ceeded the amount originally taken, the variation being from two to fifteen percent. Hence the method, at least in the form here described, is not reliable.

2. Extraction of alkaloids by means of Lloyd's reagent.

Owing to the facility and completeness of precipitation of alkaloids by Lloyd's reagent, it was thought that this reagent could be advantageously used for the quantitative extraction of alkaloids from their original sources or from the solution of their salts in water.

It is evident that, in order to attain this aim, it is necessary to prove that the alkaloids, once precipitated by Lloyd's reagent, can be readily and completely recovered from the precipitate containing alkaloid and reagent. With a view of determining this point, the following experiments were carried out:

A definite amount of morphine was dissolved in an excess of dilute sulfuric acid, the alkaloid completely precipitated with an excess of Lloyd's reagent, and the precipitate then washed with water until the washings gave no test for sulfuric acid. The precipitate was dried at 60° and then repeatedly extracted with boiling methyl alcohol, which is a very fair solvent for morphine. The solution was evaporated to dryness, and the residue weighed. This residue was free of sulfuric acid, showing it to contain probably free morphine, but its amount was less than 4 percent. of the morphine originally taken.

The precipitate was again extracted with methyl alcohol to which a small amount of ammonia had been added, and after again evaporating the solvent the residue was weighed. The total amount of alkaloid recovered by the two successive extractions, was about 90 percent. of the morphine taken.

Another experiment was made with strychnine, using chloroform which is an exceptionally good solvent for this alkaloid. A dilute solution of strychnine in water acidified with sulfuric acid, was completely precipitated with an excess of Lloyd's reagent, and the precipitate, after thorough washing with water, dried at 60°. A portion of the precipitate containing about 0.2 g. of strychnine was suspended in a little water containing an excess of ammonia, and then repeatedly shaken out with successive portions of chloroform, using 20 cc. of the latter for the first shaking and 15 cc. each time afterwards. It was found that, even after ten consecutive operations, the chloroform did not remove all of the alkaloid, as was shown by evaporating some of the chloroformic extract to dryness, taking up the residue with acidified water, and testing the resulting solution with Mayer's and Wagner's reagents, both of which continued to give a heavy precipitate. Hence, by this method, it is extremely difficult, quantitatively, to recover the strychnine from a solution of its salts in water. Whether other methods would be more successful, will have to be determined by further experimentation.

3. Attempt to facilitate the removal of strychnine from the precipitate obtained by adding Lloyd's reagent to a solution of a salt of the alkaloid in water.

The precipitate obtained by adding an excess of Lloyd's reagent to an aqueous solution of a salt of strychnine, is almost perfectly tasteless, though it contains all of the alkaloid of the original solution. This seems to suggest the view that the reagent forms with the alkaloid, an exceptionally stable combination, and this

view is further strengthened by the fact that, as was shown above, it is extremely difficult to completely recover the alkaloid from the precipitate.

On the other hand, as will be reported later by Dr. McGuigan, the precipitate acts physiologically very much like strychnine diluted with an inactive substance, showing that, in the living digestive apparatus, the union of alkaloid and reagent is readily disrupted. Since it was reasonable to ascribe this disrupting effect to the digestive enzymes of the animal body, experiments were made, in order to determine whether some of these enzymes would show the same disrupting effect *in vitro*. If this were so, dilute hydrochloric acid in presence of pepsin, or chloroform in presence of alkali and either ptyalin or trypsin, readily ought to extract the strychnine from the precipitate. The following experiments were, therefore, carried out with these enzymes:

Pepsin. The thoroughly washed and dried precipitate obtained by adding an excess of Lloyd's reagent to an aqueous solution of strychnine sulphate, was digested with very dilute hydrochloric acid containing a little pepsin, shaking the mixture for an hour and then filtering. The filtrate was tested with Mayer's and Wagner's reagents. Neither of these gave any indication of the presence of an alkaloid. Hence *in vitro* pepsin has no disrupting effect on the precipitate.

Ptyalin and trypsin. The precipitate was suspended in a very dilute solution of ammonia containing either ptyalin or trypsin, and the mixture repeatedly shaken out with chloroform. It was found that, even after ten successive treatments with chloroform, the precipitate still retained some of the strychnine. Hence these enzymes, too, have no disrupting effect on the precipitate.

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OIL OF BIRCH AND METHYL SALICYLATE,—SOME NEW COLOR-REACTIONS FOR THE DIFFERENTIATION OF OIL OF WINTERGREEN.

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Anyone who has had experience with oil of wintergreen and the synthetic oil, knows of the uncertainty connected with their identification and differentiation. The physical constants, with one exception, appear to be of little value in distinguishing the true from the artificial oil. We have the authority of C. L. Alsberg that at present, except for the one test—the presence or absence of optical activity,—there has been nothing published which would enable one to make the differentiation, and that this polarization method is only a very important factor to this end. During the past winter, at the drug laboratory, we have had occasion to examine several samples of oil of wintergreen, which brought to our attention the desirability of confirmatory tests. After numerous attempts to fix upon one, it was finally decided that rotatory power of the natural oil was perhaps the only distinguishing characteristic. Admixture with corresponding oils, such as betula and methyl salicylate, being suspected by any great digression of optical activity. Recently I have used some color-reactions which seems to promise excellent re-