washed free from chlorides, then washed with alcohol and ether and finally dried *in vacuo*.

Calculated for $C_6H_8O_{10}Bi_2$: Bi = 63.53%.
Found: Bi = 63.50%.

Sodium Di-Bismuthyl Saccharic Acid.—An attempt to prepare sodium di-bismuthyl saccharate by the same procedure used for the preparation of potassium di-bismuthyl saccharate yielded a white solid which after precipitation with alcohol was no longer soluble in water. The sodium salt, however, was made directly from di-bismuthyl saccharic acid. Ten grams of the acid as prepared above were suspended in water and 10% sodium hydroxide added until the acid just dissolved. This was then precipitated with alcohol three times as in the preparation of the potassium compound, washed with alcohol and ether and dried *in vacuo*. Analyses indicated however that the product was the acid salt.

Calculated for $C_6H_7O_{10}Bi_2Na.H_2O$: Bi = 59.89; Na = 3.29.
Found: Bi = 61.48; Na = 3.60.

Sodium Potassium Di-Bismuthyl Saccharate.—Twelve and four-tenths grams ($^1/_{20}$ mole) of potassium acid saccharate were just neutralized with sodium hydroxide and then 25 cc. excess of 25% sodium hydroxide added. To this was added freshly precipitated bismuth hydroxide from 48.5 Gm. ($^1/_{10}$ mole) of bismuth nitrate and the product precipitated with alcohol and purified in the same manner as the di-potassium compound. The resulting white compound was dried *in vacuo*.

Calculated for $C_6H_7O_{10}Bi_2NaK.H_2O$: Bi = 56.80; Na = 3.12.
Found: Bi = 59.14; Na = 3.32.

Di-Potassium Tri-Bismuthyl Saccharate.—Seventeen cubic centimeters of a 60% solution of saccharic acid ($^1/_{20}$ mole) were just neutralized with potassium hydroxide and 50 cc. of 25% solution added in excess. To this was added freshly precipitated bismuth hydroxide from 72.7 Gm. ($^3/_{20}$ mole) of bismuth nitrate in 200 cc. of water. The mixture was shaken for 48 hours as described by Kober (7), filtered and the bismuth complex precipitated with alcohol and purified in the same manner as the previous compounds. The resulting white compound appeared to be less stable than the di-bismuthyl compounds and turned brown when warmed *in vacuo*. It was dried at room temperature over calcium chloride.

Calculated for $C_6H_5O_{11}Bi_3K_2.2H_2O$: Bi = 63.06; K = 7.86.
Found: Bi = 63.13; K = 7.05.

An attempt was also made to prepare a compound containing 4 atoms of bismuth per mole of saccharic acid using the same procedure as in the preparation of the tri-bismuthyl saccharate but with a large excess of bismuth hydroxide. The resulting compound contained only 63% bismuth. It is apparently impossible to prepare potassium bismuthyl saccharates with more than 3 atoms of bismuth per mole of saccharic acid.

SUMMARY

1. Methods are described for preparing di-potassium di-bismuthyl saccharate, di-bismuthyl saccharic acid, mono-sodium di-bismuthyl saccharate, sodium potassium di-bismuthyl saccharate and di-potassium tri-bismuthyl saccharate.

2. Potassium bismuthyl saccharate has been shown to be a more stable complex than the corresponding tartrate and gluconate.

REFERENCES

(1) Warren, L. E., *J. A. M. A.*, 84 (1925), 1067.
(2) Corfield and Adams, *Chemist and Druggist*, 101 (1924), 135.
(3) Rosenheim, A., *et al.*, *Z. Anorg. Chem.*, 200 (1931), 176.
(4) Browning, *et al.*, *Proceed. Royal Soc.*, (B) 102 (1927), 1.
(5) Maschmann, E., *Arch. d. Pharm.*, 263 (1925), 99.
(6) Hepner and Likiernik, *Arch. d. Pharm.*, 264 (1926), 46.
(7) Kober, P. A., U. S. Patent 1,663,201 (1928); *Am. J. Syphilis*, 15 (1931) 199.
(8) Lauter, W. M., and Braun, H. A., JOUR. A. PH. A., 25 (1936), 497–499.
(9) Von Oettingen, W. F., Sollmann, T., and Schweid, H. H., *Ibid.*, 17 (1928), 540.
(10) Boeseken, J., *Rec. Trav. Chim.*, 40 (1921), 553, and subsequent papers.
(11) Vermaas, N., *Ibid.*, 51 (1932), 67.

Studies on Cantharides

II. The Assay of Cantharides*

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INTRODUCTION

It is generally acknowledged that the present pharmacopœial process for the assay

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of Cantharides is not without fault. It is time-consuming and does not give uniform results. Extraction of cantharidin from the crude drug is incomplete with some solvents. The volatility of cantharidin has also received too little consideration. Although cantharidin has a high melting point (218°) it is easily volatile at lower temperatures and Reimers (1) has shown losses to occur upon drying above 60°. It is even more volatile in the presence of solvent vapors.[1] Finally, the method of purification is faulty; purification of the extracted cantharidin by the official process is incomplete and the final residue which is weighed does not accurately represent the amount of cantharidin extracted.

A colorimetric determination (2) of cantharidin has not been possible; likewise, a distillation method (3) was no more successful. The idea of applying a titrimetric method to the estimation of cantharidin is not a new one, but has not been realized because it has not been shown that cantharidin is capable of being titrated. With the successful titration of this compound, as reported in the previous paper (4), it was now possible to attempt an application of this method in the improvement of the present official assay process. Since extraction, evaporation and purification procedures must still be dealt with in any method, it seemed desirable to study also these aspects of the problem.

EXPERIMENTAL

Determination of Cantharidin.—By U. S. P. XI Method: A sample of Cantharides, labeled Russian Cantharides, was obtained from the E. H. Sargent Company and was assayed by the U. S. P. XI process. The results (see Table I) are expressed in per cent and also as Gm. of cantharidin representing 10 Gm. of dried drug.

The highest value found for the cantharidin content was 0.654%; the lowest was 0.384%; the mean was 0.554%. In only three cases was the amount above 0.6%, which is the minimum requirement as stated in the United States Pharmacopœia.

The reports on the cantharidin content of Russian Cantharides seem to vary considerably with different workers. There also seems to be a variance from one year to another. Patch's report (5) in 1913 listed the per cent of cantharidin in five samples of cantharides as 0.4, 0.43, 0.4, 0.44, 0.34. The report for the same year by Ewe (6) for twenty-one samples showed nine below the standard of 0.6%.

Table I.—Assay of Cantharides, U. S. P. XI Method

Sample	Gm.	Per Cent	Per Cent Deviation from Mean[a]
1	0.0572	0.572	+ 2.88
2	0.0512	0.512	− 7.58
3	0.0503	0.503	− 9.20
4	0.0654	0.654	+18.05
5	0.0637	0.637	+14.98
6	0.0571	0.571	+ 2.88
7	0.0553	0.553	− 0.18
8	0.0518	0.518	− 6.48
9	0.0593	0.593	+ 7.03
10	0.0593	0.593	+ 7.03
11	0.0596	0.596	+ 7.76
12	0.0568	0.568	+ 2.52
13	0.0484	0.484	−12.63
14	0.0633	0.633	+14.25
15	0.0582	0.582	+ 5.05
16	0.0466	0.466	−18.84
17	0.0566	0.566	+ 2.16
18	0.0572	0.572	+ 2.88
19	0.0384	0.384	−30.66
20	0.0459	0.459	−17.14
21	0.0406	0.406	−26.71
22	0.0408	0.408	−20.93
23	0.0455	0.455	−17.87
24	0.0458	0.458	−17.32

[a] Mean = 0.554%.

By Titration: Procedure: 15-Gm. samples of Cantharides were used. The extraction was carried out according to the directions of the U. S. P. XI process, with the notable exception that the evaporation of the solvent from the extract was performed in a six-inch evaporating dish instead of a tared beaker as directed by the U. S. P. The impure cantharidin obtained after the purification procedure was then dissolved in 25 cc. of acetone, and 25 cc. of $N/20$ alcoholic KOH added, followed by 25 cc. of distilled water. This was evaporated to a volume of about 10 cc., or until acetone and alcohol were no longer detectable. The excess alkali was then titrated with $N/20$ HCl, using phenolphthalein. A blank was run, using 25 cc. of acetone.

One cubic centimeter $N/20$ KOH = 0.0049 Gm. cantharidin. The number of grams of cantharidin found represents 10 Gm. of Cantharides.

Table II.—Cantharidin by Titration

Sample	Per Cent of Cantharidin	Per Cent Deviation from Mean[a]
1	0.666	− 1.62
2	0.652	− 3.69
3	0.666	− 1.62
4	0.701	+ 3.54
5	0.794	+17.28
6	0.701	+ 3.54
7	0.607	−10.33
8	0.627	− 7.38

[a] Mean = 0.677%.

The lowest value found was 0.607% of cantharidin; the highest was 0.794%; the mean for the series was 0.677%. The maximum deviation from the

[1] Note: Severe "cantharidin burns" around the face were experienced while evaporating an alcoholic solution of cantharidin.

mean was 17.28%. All results were above the minimum requirement of 0.6%, U. S. P. The large per cent of deviation in several instances requires further study.

Comparison of U. S. P. XI and Titration Processes.—Samples of Cantharides were assayed by the U. S. P. method. Each residue of cantharidin obtained from these assays was then subjected to titration as previously described. The results of each determination are listed.

Table III.—Comparison of Assay Results

Sample	Per Cent of Cantharidin by U. S. P. XI Method	Per Cent of Cantharidin by Titration Method
1	0.654	0.406
2	0.637	0.347
3	0.572	0.382
4	0.384	0.265
5	0.459	0.436
6	0.406	0.338
7	0.408	0.372
8	0.455	0.396
9	0.458	0.396

Each sample of cantharidin obtained from the assay of Cantharides by the U. S. P. process gave, on titration, smaller values. This indicates that the residue obtained in the U. S. P. process, the weight of which is directed to be called the amount of cantharidin in the drug, is not all cantharidin. This is also evident by mere inspection of the product. It is apparent that the petroleum ether and alcohol mixture, previously saturated with cantharidin, does not remove all of the inert material.

Investigation of the Volatility of Cantharidin.—Cantharidin was dissolved in 100 cc. of a mixture consisting of 1 volume of petroleum ether and 2 volumes of benzene. The solution was then evaporated in a tared 50-cc. beaker on a steam-bath. The time for complete evaporation of the entire solution was noted. After drying at 40° for 20 minutes, the beaker and its contents were weighed and the loss in weight sustained during the process of evaporation found.

Table IV.—Volatility of Cantharidin from Ether-Benzene

Gm. Cantharidin	Time Required for Complete Evaporation	Loss in Weight in mg.	Per Cent Loss in Weight
(*a*) 0.2220	2¼ hours	9.6	4.32
(*b*) 0.0797	1½ hours	2.0	2.50
(*c*) 0.2683	1¾ hours	4.4	1.63
(*d*) 0.2246	1½ hours	2.0	0.80

A similar solution of cantharidin was evaporated in a 6-inch evaporating dish, and, after complete removal of solvent, the cantharidin was transferred to a tared beaker using warm chloroform for this purpose. The chloroform was evaporated quickly and the cantharidin dried and weighed.

Table V.—Volatility of Cantharidin from Ether-Benzene, then Chloroform

Gm. Cantharidin	Time required for Complete Evaporation	Loss in Weight in mg.	Per Cent Loss in Weight
(*a*) 0.1556	8 minutes	0.1	0.06
(*b*) 0.1901	6 minutes	0.2	0.10

In the U. S. P. assay process extraction is carried out with a mixture of petroleum ether and benzene, and this solvent is then evaporated. In carrying out this procedure a small tared beaker is ordinarily used, as the cantharidin is directed to be weighed after purification. The time required for this operation, employing a steam-bath, is about two hours. In the above experiment, using the same solvent and evaporating in a 50-cc. beaker, there was a definite loss of cantharidin. In the second part of the experiment, where evaporation extended over minutes only, the loss was negligible. In the actual assay process by the U. S. P. method, there should be even greater loss of cantharidin, as the presence of extracted fat and other matter should increase the boiling point of the solvent. In the titration method, evaporation is carried out in a six-inch evaporating dish and this process usually does not require more than ten minutes. This helps to explain the higher values obtained by the titration process and its advantage over the U. S. P. process.

Assay of Cantharides.—Using Chloroform for Extraction: As chloroform is an excellent solvent for cantharidin, Cantharides was extracted with this solvent and the titration assay applied. The results from these assays would then serve as an index to the thoroughness of extraction by other solvents, especially the one used in the official process. The extraction procedure was carried out according to the U. S. P. XI directions, using a solvent of 150 cc. of chloroform and 2 cc. of hydrochloric acid. The chloroform was distilled from the extract, the impure residue purified as in the U. S. P., and the final residue of cantharidin then titrated by the method previously outlined.

Table VI.—Assay of Cantharides Using Chloroform Extraction

Sample	Per Cent Cantharidin Found	Per Cent Deviation from Mean[a]
1	0.652	+ 4.48
2	0.588	− 5.76
3	0.627	+ 0.48
4	0.681	+ 9.13
5	0.676	+ 8.33
6	0.519	−16.82

[a] Mean = 0.624%.

The lowest value found was 0.519%; the highest was 0.681%; the mean was 0.624%. The mean of this series is somewhat lower than that found in the previous series in which extraction was made with the official solvent, benzene and petroleum ether. These results indicate that extraction is complete with either solvent, but that the differences may be due to some other factor.

Using Petroleum Ether to Remove Fat: In the U. S. P. method, a mixture of petroleum ether and dehydrated alcohol, previously saturated with cantharidin, is employed to purify the extracted cantharidin. The petroleum ether serves to remove fat and coloring material, while the alcohol removes resin-like material.

Fifteen-gram samples of Cantharides were extracted as previously described following the U. S. P. procedure, using a mixture of two volumes of benzene and one volume of petroleum ether with 2 cc. of hydrochloric acid added. The residue, obtained after filtering off 100 cc. of the extract and evaporating, was washed with small portions of petroleum ether to remove fat and coloring material. The impure cantharidin was then titrated by the method previously described.

Table VII.—Assay of Cantharides after Defatting

Sample	Per Cent Cantharidin Found	Per Cent Deviation from Mean[a]
1	0.593	−0.33
2	0.559	−6.05
3	0.632	+6.05
4	0.588	−1.17
5	0.583	−2.01
6	0.617	+3.69

[a] Mean = 0.595%.

For the per cent of cantharidin, the lowest value found was 0.583%; the highest was 0.632%; the mean per cent was 0.595%. Four of the results were below the U. S. P. requirement of 0.6%.

In the titration method of assay, the use of petroleum ether to remove fat and coloring matter is sufficient; the use of alcohol is entirely unnecessary. As some of the results were below the minimum U. S. P. requirement, it is evident that there must be a loss of cantharidin during the process of removing fat, especially since cantharidin is soluble in fats and oils.

Loss of Cantharidin Due to Presence of Fat.—In order to determine the extent of the loss of cantharidin due to the presence of fat and oil, the following experiments were carried out:

Weighed quantities of cantharidin were dissolved in chloroform and 5 cc. of castor oil were added. The solutions were then placed in a warm place until the chloroform was completely evaporated. The oil was then removed by washing with 10-cc. portions of a solution consisting of equal volumes of petroleum ether and dehydrated alcohol, previously saturated with cantharidin. To prevent washing away of cantharidin, the washings were filtered through cotton, and the retained cantharidin was dissolved out with warm chloroform and added to the remaining crystals. The chloroform was evaporated and the cantharidin dried at 40° for thirty minutes. It was then weighed and the amount of cantharidin lost was found.

Table VIII.—Loss of Cantharidin Due to Presence of Fat

Sample	Gm. of Cantharidin Treated	Mg. of Cantharidin Lost	Per Cent Lost
1	0.2476	23.0	9.28
2	0.0960	19.6	20.41

The above experiment was repeated using petroleum ether in place of the petroleum ether-alcohol mixture for washing out the fat.

While this experiment was performed with castor oil, which may not have the same characteristics as the fat actually present in Cantharides, it nevertheless illustrates that there is considerable loss of cantharidin during the process of removing fat. This factor helps to cause the low or uneven results in both the U. S. P. and titration processes. Any successful method of assay must therefore take into consideration this factor and prevent such loss.

Table IX.—Loss of Cantharidin after Washing Out Fat

Sample	Gm. of Cantharidin Treated	Mg. of Cantharidin Lost	Per Cent Lost
1	0.2892	20.9	7.22
2	0.0967	13.7	14.16

Attempt to Assay Cantharides with Removal of Fat from Alkaline Solution.—If it were possible to convert the cantharidin present in the extract into an alkali salt of cantharidic acid, the fat and other impurities could be removed from this salt by shaking it out in aqueous solution with chloroform, acidifying the fat-free solution and extracting the cantharidin with chloroform. In this way the loss of cantharidin might be prevented. Accordingly, this possibility was investigated, but the troublesome emulsions encountered in attempting to shake out the alkaline solution with chloroform made this method impracticable.

Assay of Cantharides Using Alcohol for Extraction.—As alcohol is not a good solvent for fat, this was used to extract Cantharides and the assay applied. It was recognized that alcohol is also a poor solvent for cantharidin (1:11,500) but, supposedly, enough solvent was used in the process to completely extract it. Furthermore, the presence of hydrochloric acid in the menstruum would be expected to increase the solvent power for cantharidin.

Table X.—Assay of Cantharides Using Alcohol for Extraction

Sample	Per Cent Cantharidin Found
1	0.299
2	0.309

While the process was easily carried out, the results of the assay show that extraction of cantharidin with alcohol is very incomplete, making further work with this solvent useless.

Assay of Tincture of Cantharides.—Procedure: 100 cc. of tincture prepared by the U. S. P. XI process were made alkaline with a 20% solution of potassium hydroxide and heated on a steam-bath until the alcohol was removed. The remaining portion was cooled and then filtered through cotton into a separatory funnel. It was then shaken out with 10-cc. portions of chloroform until the washings were colorless. The aqueous solution was made acid to congo red with hydrochloric acid and extracted with 10-cc. portions of chloroform until all cantharidin was dissolved out. The chloroform was

evaporated and the residue dried. The impure cantharidin was washed twice with petroleum ether (5 cc.) to remove possible traces of fatty acids. By the previously outlined method, the cantharidin residue was titrated and the amount determined.

Table XI.—Results Obtained in the Assay of Tincture of Cantharides

Sample	Gm. of Cantharidin in 100 Cc. of Tincture	Per Cent Deviation from Mean[a]
1	0.0236	− 4.83
2	0.0265	+ 6.85
3	0.0274	+10.48
4	0.0245	− 1.22
5	0.0221	−10.88

[a] Mean = 0.0248 Gm.

SUMMARY AND CONCLUSIONS

1. A number of samples of Cantharides have been assayed according to the U. S. P. XI method. It was found that most of these were below the U. S. P. standard of 0.6%. These low results were shown to be due partially to losses of cantharidin, due to its volatility, during the evaporation procedure of the assay, and partially to the method used for the removal of fat from the extracted cantharidin. It was shown that the residue, obtained in the process and weighed as cantharidin, is not all cantharidin, but contains much inert material that is difficult to separate.

2. An improvement on the U. S. P. method has been suggested and tried out. This method requires only a very short period of time for evaporation, as compared to the official method, and determines the cantharidin content of the extracted residue by titration. Although this method yields results higher than the U. S. P. requirement, it has the advantages of being less tedious and time-consuming, and overcomes the loss of cantharidin due to volatilization. It does not overcome the loss of cantharidin sustained in the removal of fat from the extracted residue.

3. In a study of the different parts of the U. S. P. method it has been shown that the solvent of benzene and petroleum ether is satisfactory for the complete extraction of cantharidin. Chloroform was also shown to extract it completely; alcohol was shown to be a poor, unsatisfactory solvent.

4. Attempts to improve the manner in which fats and inert material are removed from the extracted residue were unsuccessful. This part of the assay procedure is in need of further work.

5. Preliminary results on the application of the titration method for estimating the cantharidin content of the tincture are reported. It is recommended that this method is worthy of further investigation for the assay of both the drug and the tincture.

REFERENCES

(1) Reimers, F., *Arch. Pharm.*, 249 (1911), 259.

(2) Listroy and Denise, *J. pharm. Belg.*, 15 (1933) 1.

(3) Rosenthaler, L., *Nachweis der Organischen Verbindungen* (1914), page 834.

(4) Hecht, B. J., and Parks, L. M., JOUR. A. PH. A., 29 (1940), 71.

(5) Patch, E. L., *Ibid.*, 2 (1913), 1088.

(6) Ewe, G. E., *Ibid.*, 9 (1920), 261.

The Separation and Determination of Aminopyrine in Mixtures

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INTRODUCTION

The use of synthetic organic substances in medicine is rapidly increasing. During the past two or three decades, the tendency to mix two or more of the synthetics in a single dose has been in evidence. Such mixtures as acetophenetidin, acetylsalicylic acid and caffeine, acetophenetidin and salol, aminopyrine and phenobarbital, aminopyrine and caffeine, and acetylsalicylic acid and phenobarbital are not uncommon. Occasionally, as many as four active medicinal ingredients are found in a single dosage form (acetophenetidin, aminopyrine, caffeine and barbital or phenobarbital).

Satisfactory methods are not available for separating aminopyrine from the mixtures in which it is found. Due to the numerous inquiries which have been received by the Food and Drug Administration, an attempt

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