6. EXTRACTION OF THE ALKALOIDS FROM CINCHONA FEBRIFUGE AND TOTAQUINA.

Introduction	138
Experimental	138 - 141
Results and Discussion	141 - 149
Conclusion	149 - 150
References	150

6. EXTRACTION OF THE ALKALOIDS FROM CINCHONA FEBRIFUGE AND TOTAQUINA :

Introduction :

In this chapter, studies on extraction of the alkaloids from cinchona febrifuge and totaquina were made using the resin IR-200.

Experimental :

Chemicals : All the chemicals used were of C.P.grade.

The study of this chapter was divided into two parts.

Part I

Cinchona febrifuge :

This was supplied by the Government Quinine Factory, West Bengal. The alkaloid material was analysed as follows :(1-2)

Two grams of the cinchona febrifuge were dissolved in N sulfuric acid (20 cc.), distilled ethyl alcohol (40 cc.) and water (40 cc.). This was heated to boiling, N/10 sodium hydroxide was added, keeping the liquid hot during the addition, until the solution was just faintly alkaline to solution of litmus. It was allowed to cool, N/10 sulfuric acid was added drop by drop, until the solution was slightly acid to litmus. It was then boiled for about two minutes, cooled, again rendered slightly acid to litmus, if necessary, boiled and filtered into a tared flask. The original vessel and the filter were washed with the boiling water until

complete extraction of the alkaloids was effected, adding the washings to the original filtrate. The filtrate was evaporated until it weighed about 120 gms. Powdered sodium potassium tartarate (30 gms.) was added and was allowed to stand for 24 hours. The precipitate was filtered through a hardened filter, the flask and the filter were washed with 25 % W/V solution (80 cc.) of sodium potassium tartarate in water, added in portions. The filtrate and washings were preserved. The filter with the precipitate was transferred to a flask, 20 % sodium hydroxide (40 cc.) and chloroform (80 cc.) were added, allowed to stand, shaking from time to time, until complete solution was effected. The chloroform solution was separated, the flask and the aqueous liquid were washed with a little water. The chloroform layer was removed into a weighed flask, distilled ethyl alcohol (5 cc.) was added, evaporated on a water bath, dried at 100 ± 1^{o} C and finally weighed. The difference in weights gave the amount of quinine and cinchonidine in the mixture. Distilled ethyl alcohol (100 cc.) was added into the same flask, the mixture of quinine and cinchonidine was dissolved and the amounts of quinine and cinchonidine were estimated by studying the ultraviolet absorption at 332 mμ (3) after suitable dilution with ethyl alcohol.

The filtrate and washings preserved earlier were run into a separator containing ether (80 cc.) and 20 % W/V sodium hydroxide solution (20 cc.) and shaken well. The aqueous layer was run into a second separator, shaken with

two further quantities of ether (80 cc.), each quantity of ether being returned to the first separator. The mixed etherial solution was washed with a little water and the alkaloids were extracted by shaking with successive quantities of N sulfuric acid (10,10 and 5 cc.) and finally with water (10 cc.). The mixed acid and aqueous liquids were run into a separator containing ether (25 cc.) and N sodium hydroxide solution (30 cc.), shaken well and were allowed to stand for an hour. The precipitated cinchonine was collected on a tared filter, using a little water to facilitate the complete transfer of the precipitate to the filter ; the ether from the filter was separated, it was again run through the precipitate on the filter. The aqueous liquid was again shaken with two separate quantities of ether (25 cc.) and these etherial washings were used to wash the precipitate. This precipitate of cinchonine was dried at 100 $\pm$ 1 ^{o}C and weighed. To this weight 0.08 gm.was added in order to correct for the loss of cinchonine due to its solubility in ether.

The etherial filtrate from the cinchonine was run into a separator ; the filter flask was washed with a little water and ether and the washings were added to the liquid in the separator. The aqueous layer was separated and the alkaloid from the etherial solution was extracted by shaking with successive quantities of 10 % W/W aqueous solution of glacial acetic acid (10,10,5 and 5 cc.) which had been previously used to wash out any alkaloid remaining in the

filter flask or on the stem of the funnel. The mixed acid solution was heated to boiling point, neutralised with dilute solution of ammonia, potassium iodide (5 gms.) was added, allowed to stand over night, the supernatent liquid was decanted through a filter, the precipitate was warmed with 50 % ethyl alcohol (5 cc.), the liquid was filtered off and 50 % ethyl alcohol (5 cc.) was passed through the crystalline residue. It was then dried at 100 $\pm$ 1 ^{o}C and weighed. To the weight of quinidine hydriodide thus obtained 0.008 gm. was added to correct for the loss of quinidine hydriodide due to its solubility. Each gram of quinidine hydriodide is equivalent to 0.717 gm. of quinidine.

The analysis based on the alkaloid material was : quinine = 21 % , quinidine = 23.5 % , cinchonine = 9.4 % , cinchonidine = 7 % and the other material (by difference) = 39.1 %.

Results and discussion :

To study the extraction of the alkaloid material from cinchona febrifuge six runs were carried out using columns of resin IR-200 in hydrogen, sodium and ammonium forms.

Run one :

A column of resin IR-200 in hydrogen form was set up. The column data were :
bed length = 56 cms., bed volume = 137 cc., capacity of the resin in the column = 265 meq.

Cinchona febrifuge (15 gms.) was put into a beaker containing 5 liters of N/100 sulfuric acid (pH = 2.2 - 2.3), kept under constant stirring for four hours, allowed to settle and filtered. The filtrate was taken in an aspirator and passed through the resin column. The flow rate was about 15 cc. per minute. The effluent was collected in a beaker, its pH was adjusted to the original value and the febrifuge on the filter paper was transferred to the same beaker along with the filter paper, stirred well, filtered and once again passed through the same resin column. This was continued till the alkaloids were removed from the material (indicated by the absence of the fluorescence). Then the column was washed with distilled water, backwashed to remove the insoluble material, if any, from the resin bed, the exchanged resin was transferred into a beaker containing N sodium hydroxide (500 cc.) and allowed to stand over night. The resin with the liberated alkaloids was filtered under vacuum, excess alkali was washed with a little distilled water and the filter paper with the resin and the liberated alkaloid bases were transferred to another beaker containing distilled ethyl alcohol (500 cc.), allowed to stand for an hour with frequent stirring and filtered through a sintered glass funnel. The resin was again transferred to the first beaker, N sodium hydroxide (300 cc.) was added again and the extraction of the liberated alkaloid bases were done in the same way as before. This was continued until the alkaloids were completely removed from the resin particles (determined by evaporating 1 cc.of the

extracte(alcoholic) in a test tube). The alcoholic extract was concentrated by distillation, evaporated on a water bath, dried at 100 ± 1 °C and weighed. The amount of the alkaloid mixture obtained was 9.0 gms. Hence the yield is 60 % on the basis of the raw material. The mixture was then analysed as described earlier and the analysis gave : quinine = 1.49 gms., quinidine = 2.84 gms., cinchonine = 1.35 gms., cinchonidine = 0.45 gms., and other material = 2.86 gms.

Run two :

This was the repetition of run one except that the column was in sodium form instead of in hydrogen form. The amount of the alkaloid materialsobtained and its analysis were almost the same as given for run one.

Run three :

This was the repetition of the run one except that in this case 40 gms. of febrifuge were taken instead of 15 gms. The acid extract of the alkaloid material was passed till considerable leakage occured from the resin column (determined by the fluorescence of the effluent). The amount of the alkaloid mixture obtained was 19.0 gms. This corresponds to 20 % of the resin capacity of the column. The analysis of the alkaloid mixture gave : quinine = 3.13 gms., quinidine = 6.5 gms., cinchonine = 3.32 gms., cinchonidine = 1.05 gms. and the other material (by difference) = 5.0 gms.

Run four :

This was the repetition of run three except that the column was converted into sodium form before the exchange run. The amount of the alkaloid mixture obtained was 19.0 gms. and its analysis was almost the same as given in run three.

Run five :

The above column was regenerated with the excess of hydrochloric acid, washed free of acid and converted into ammonium form by passing excess of ammonium hydroxide solution. The column was then washed free of ammonia, backwashed and allowed to settle under gravity. 40 gms. of febrifuge were dissolved by stirring in 5 liters of N/100 sulfuric acid and was exchanged with the column as described earlier. When considerable leakage of the alkaloids from the column occured, the column was washed, backwashed, allowed to settle under gravity and the exchanged alkaloids were eluted by passing N/10 ammonical ethyl alcohol at a rate of 15 cc. per minute. The elution was continued till all the alkaloids were removed from the resin bed (determined by evaporating 1 cc. of the effluent in a test tube). The alkaloid extract was concentrated by distillation, evaporated on a water bath, dried at 100 $\pm$ 1 ^{o}C and finally weighed. The total amount of the alkaloid mixture obtained was 19.0 gms. This corresponds to 20 % of the resin capacity of the column.

The analysis of the mixture gave :
quinine = 3.14 gms., quinidine = 6.55 gms.,
cinchonine = 3.31 gms., cinchonidine = 1.06 gms. and
the other material = 4.94 gms.

Run six :

This was the repetition of run five except that after the exchange run the column was washed with five bed volumes of 50 % aqueous ethyl alcohol. This removed a good part of the coloring matter. After this the alkaloid material was eluted with N/10 ammonical ethyl alcohol, the alkaloid extract was concentrated and was kept over night in a refrigerator. The crystallised alkaloid material was filtered, dried at 100 ± 1 °C and weighed. This is further referred to as yield I. The filtrate was evaporated on a water bath, dried at 100 ± 1 °C and weighed. This is further referred to as yield II. The amounts of the alkaloid mixture in yield I and yield II were 12 gms. and 7 gms. respectively. Their analysis gave :
quinine = 1.00 gm., quinidine = 3.06 gms.,
cinchonine = 3.1 gms., cinchonidine = 0.67 gm. and
the other material = 4.17 gms. for yield I and
quinine = 2.08 gms., quinidine = 3.64 gms.,
cinchonine = 0.25 gm., cinchonidine = 0.40 gm. and
the other material = 0.63 gm. for yield II.

The analysis of the febrifuge for individual alkaloids indicates that it contains about 60 % of the

four alkaloids quinine, quinidine, cinchonine and cinchonidine. In run one were 15 gms. of the febrifuge were taken for exchange with the resin column the yield obtained is 9 gms. This corresponds to 60 % of the raw material taken. The analysis of the extracted material (9 gms.) indicates that it contains 6.14 gms. of the four alkaloids and this corresponds to 68.2 % of the extracted material and 41 % of the raw material taken. This indicates that the complete extraction of the four alkaloids did not take place. In [illegible] runs 3 to 6 40 gms. of raw material give 19 gms. of the extracted alkaloids. This 19 gms. contains 14 gms. of the four alkaloids which corresponds to 73.7 % of the extracted material. The results also indicate that the yield and its analysis are independent of the ionic form of the resin and of the eluent. The use of 50 % ethyl alcohol for washing the exchanged resin bed before the extraction of the alkaloids with N/10 ammonical ethyl alcohol helps in removing a good part of the coloring matter and the product is relatively much less colored than those obtained from other runs.

Part II

This part includes similar studies with totaquina.

Totaquina :

This was supplied by the Government Quinine Factory, West Bengal. The analysis of this alkaloid containing

material was done in the same way as described in Part I for the analysis of the cinchona febrifuge. The analysis gave :

quinine = 28.5 % , quinidine = 30.2 % , cinchonine = 14.5 % , cinchonidine = 10.6 % and the other material (by difference) = 16.2 %.

Run one :

This was the repetition of run one of Part I except that the alkaloid material taken was totaquina instead of cinchona febrifuge. The amount of the alkaloid mixture obtained was 13.0 gms. and its analysis gave :

quinine = 3.25 gms., quinidine = 5.59 gms., cinchonine = 2.28 gms., cinchonidine = 1.17 gms. and the other material = 0.71 gm.

Run two :

This was the repetition of run two of Part I with totaquina instead of cinchona febrifuge. The amount of the alkaloid mixture obtained and its analysis were almost the same as given for run one.

Run three :

This was the repetition of run one except that the amount of totaquina taken was 40 gms. instead of 15 gms. The total amount of the alkaloid mixture obtained was 19 gms. and it analysis gave :

quinine = 5.32 gms., quinidine = 8.13 gms.,
cinchonine = 3.42 gms., cinchonidine = 1.90 gms.and
the other material 0.33 gms.

Run four :

This was the repetition of run three except that the column was converted into sodium form before the exchange run. The amount of the alkaloid mixture obtained and its analysis were almost the same as given for run three.

Run five :

This was the repetition of the run five of Part I except that the alkaloid material taken was totaquina instead of cinchona febrifuge. The amount of alkaloid mixture obtained was 19 gms. and its analysis gave :
quinine = 5.31 gms., quinidinine = 8.10 gms.,
cinchonine = 3.38 gms. cinchonidine = 1.87 gms. and
the other material = 0.34 gm.

Run six :

This was the repetition of run six of Part I with totaquina. The amounts of the alkaloid mixture obtained in yield I and yield II were 6 gms. and 13 gms.respectively. Their analysis gave :
quinine = 0.42 gms., quinidine = 1.56 gms.,
cinchonine = 3.20 gms., cinchonidine = 0.68 gm.and
the material = 0.14 gm. for the yield I and

quinine = 4.83 gms., quinidine = 6.60 gms., cinchonine = 0.28 gm., cinchonidine = 1.09 gms. and the other material = 0.20 gm. for the yield II.

The analysis of totaquina indicates that it contains 83.8 % of the four alkaloids quinine, quinidine, cinchonine and cinchonidine. In run one where 15 gms.of the totaquina were taken for exchange with the resin column the yield obtained is 13 gms. This corresponds to 86.6 % of the crude alkaloid material taken. The analysis of this gave 12.3 gms. of the four alkaloids and this corresponds to 81.9 % of the crude material taken indicating that 98 % of the four alkaloids are extracted. From runs 3 - 6 of the Part II, it is seen that the yield and its analysis are almost independent of the ionic form of the resin and the yield is about 20 % of the resin capacity. The data obtained with cinchona febrifuge and totaquina indicate that when large excess (40 gms.) of the raw material was taken relative to the resin capacity the yield (19 gms.) of the alkaloid mixture obtained corresponds to 20 % of the resin capacity. As in the case of cinchona febrifuge, it was found that the alkaloid extract obtained in the run six was relatively less colored than those obtained with previous runs.

Conclusion :

From the results obtained in Part I and Part II it may be concluded that a column of resin IR-200 in

ammonium form may be used with N/100 sulfuric acid as the extraction solvent and 50 % ethyl alcohol followed by N/10 ammonical ethyl alcohol as the eluent to recover the alkaloids from alkaloid containing materials such as cinchona febrifuge and totaquina. A series of four columns may be used in such a way that at any time three columns are in the exchange run and the exhausted fourth one is under elution. In this way the process may be made continuous.and a yield of about

References :

1. Pharmacopoeia of India, first edition, Government of India, Ministry of Health, 142-43 (1955).
2. Garratt, The Quantitative Analysis of Drugs,third edition, Chapman and Hall, 173-77 (1964).
3. Kamath, B. R., Bhat, C. V. and Bafna, S. L., Indian J. Chem., accepted.