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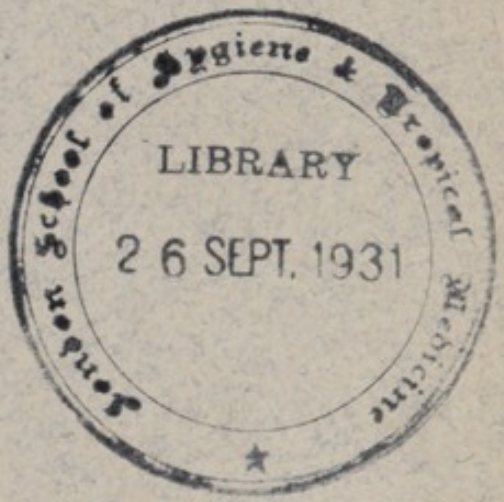
J. A. GOODSON AND T. A. HENRY

(*From the Pharmaceutical Journal*, 1930)

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# The Assay of Mixtures of Cinchona Alkaloids

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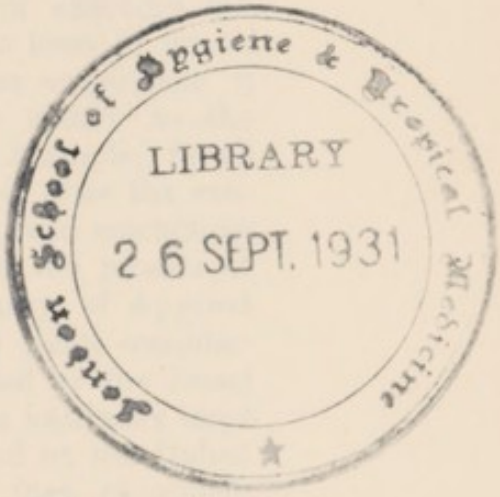


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# THE ASSAY OF MIXTURES OF CINCHONA ALKALOIDS.

Prof. P. Biginelli and Dr. F. Scordia have published recently (*Rivista di Malariologia*, 1929, Vol. 8, p. 534) a method for the estimation of quinine and quinidine together in mixtures of cinchona alkaloids, depending on the fact that these two alkaloids contain a methoxyl group, whilst cinchonine and cinchonidine do not. The quantity of methoxyl present is determined in the usual way by Zeisel's method. The following are some examples of their results with commercial and other samples of cinchona alkaloids :—

| | |
|---|---|
| Quinine, specially purified | 98·4 per cent. of quinine |
| Quinidine, laboratory stock | 91·6 per cent. of quinidine |
| Cinchonidine, ,, ,, | 3·6 per cent. of quinine and quinidine |
| Cinchonine, commercial ... | 2·1 per cent of quinine and quinidine |
| Quinetum (red colour) ..... | 37·1 per cent. of quinine and quinidine |
| Quinetum (green colour) ... | 44·2 per cent. of quinine and quinidine |

Methoxyl determination as a method of checking the results of assays of cinchona alkaloids has been in use in these laboratories since 1921, and in our experience it cannot with safety be applied quite so widely as the Italian authors suggest. We have, for example, found that the residue of amorphous alkaloids left after the estimation of quinine, cinchonidine, quinidine, and cinchonine in the total alkaloids of cinchona bark by the process of Howard and Chick (Thorpe's 'Dictionary of Applied Chemistry,' 2nd Edition, Vol. II., p. 261) yields considerable amounts of methyl iodide when treated by the Zeisel method, and this no doubt accounts for the unusually large amounts of "quinine and quinidine" found by the Italian authors in the samples of "quinetum" they examined. Similarly a sample of "quinoidine," which is supposed to consist of the uncrystallisable bases of cinchona, yielded methyl iodide equivalent to 4·7 per cent. of methoxyl, which would correspond to the impossible quantity of 48·8 per cent. of "quinine and quinidine" on the basis adopted by the Italian authors. Nevertheless, the estimation of methoxyl properly applied is a useful addition to the methods available for the assay of mixed cinchona alkaloids, and it may be of interest to place on record one example of the way in which we have found it of value.

## Cinchona Barks from Tanganyika Territory.

A number of samples of cinchona bark from this territory (formerly German East Africa) grown in the neighbourhood of Amani have been examined in recent years. A summary of these results is given in the following table :—

| | *C. Ledgeriana* | | *C. succirubra.* | | *C. robusta* | *C. succirubra* × *C. Ledgeriana.* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. | Per cent | Per cent. | Per cent. | Per cent. |
| Moisture ..... | 8·9 | 10·9 | 7·9 | 8·8 | 8·1 | 7·5 | 7·8 | 7·3 | 7·3 |
| Total alkaloids | 5·0 | 4·5 | 8·3 | 5·8 | 7·6 | 11·3 | 10·2 | 10.3 | 11·8 |
| Quinine ..... | 3·8 | 3·4 | 2·5 | 1·8 | 2·7 | 8·4 | 6·9 | 5·4 | 6·7 |
| Cinchonidine ... | nil | — | 2·05 | — | 3·5 | nil | 0·6 | 2·7 | 0·7 |
| Quinidine ..... | — | — | — | — | — | — | nil | nil | nil |
| Yield of crystallised quinine sulphate ..... | 5·1 | 4·6 | 3·4 | 2·4 | 3·5 | 11·2 | 9·5 | 7·4 | 9·2 |

Samples 1 to 6, examined at the Imperial Institute, *Bulletin of the Imperial Institute*, 1918, Vol. 16, p. 387; and 1920, Vol. 18, p. 22.

Samples 7 to 9, examined by Greenish and Corfield, *Year Book of Pharmacy*, 1923, p. 652.

Five samples of cinchona bark from the same territory have also been examined in these laboratories, two, said to be *Cinchona succirubra* bark, received from Dr. Gallagher, of the West Africa Medical Service, in 1921, and three, sent in 1923 by Mr. Alleyne Leechman, of the Amani Research Institute, as hybrid barks, presumably *C. succirubra* × *C. Ledgeriana*. The results of analysis of these five barks are given in the following table :—

| — | *C. succirubra.* | | Hybrid Barks. | | |
|---|---|---|---|---|---|
| | A. | B. | C. | D. | E. |
| | Per cent. | Per cent. | Per cent. | Per cent. | Per cent. |
| Moisture ..... | 9·0 | 9·5 | 9·8 | 9·8 | 9·6 |
| Total Alkaloids : | | | | | |
| B.P. 1914 process ... | 7·0 | 7·9 | 9·5 | 10·2 | 11·3 |
| U.S.P. 1916 process | 6·7 | 5·9 | 9·9 | 9·6 | 11·1 |
| Quinine and Cinchonidine : B.P. 1914 process..... | 5·1 | 5·9 | 7·5 | 6·9 | 8·5 |
| Ether soluble alkaloids : U.S.P. 1916 process ..... | 5·9 | 5·4 | 8·9 | 8·9 | 10·8 |
| *Quinine ..... | — | — | 6·2 | 3·8 | 6·5 |
| *Cinchonidine ..... | — | — | 1·2 | 3·3 | 1·7 |

* Determined by Howard and Chick's process (Thorpe's Dictionary of Applied Chemistry, 2nd Edition, Vol. 2, pp. 260, 266, 268).

It will be seen that these results do not differ materially from those recorded by the Imperial Institute and by Greenish and Corfield, except as regards sample D, which, though it was described as a hybrid bark and, like other hybrid barks from Amani gave a large yield of total alkaloids, differed from them in containing approximately equal amounts of quinine and cinchonidine. The total alkaloids from 3 kilogrammes of this sample were therefore prepared and further examined. They were fractionated by Howard and Chick's process and the fractions treated as follows :—

Crude quinine sulphate, Crop I., 124·9 Gms.; Crop II., 40·1 Gms.

Crude cinchonidine tartrate, 41·5 Gms.

The crude quinine sulphate, Crop I., was recrystallised, and yielded, Crop A 100·9 Gms., and a secondary crop 5·7 Gms. The crude cinchonidine tartrate was reconverted to base and the latter refractionated, yielding quinine sulphate 9·0 Gms. and cinchonidine tartrate, Crop C, 25·2 Gms. Crop A of quinine sulphate was kept separate, whilst the secondary crops of 40·1, 5·7, and 9·0 Gms., i.e., 54·8 Gms. in all, were mixed to form Crop B of quinine sulphate. Crop A reconverted to base gave in Zeisel's methoxyl process, methyl iodide equivalent to 7·075 per cent. methoxyl corresponding to 73·9 per cent. of quinine and 26·1 per cent. of cinchonidine (*by difference*).

The specific rotation of the base from Crop A was −144·9° in alcohol ($c$ = 5). Taking Rabe's figures (*Annalen*, 1910, Vol. 373, p. 89) for the specific rotation of quinine as −158·2° and for cinchonidine as −111°, this figure corresponds to quinine 71·8 per cent. and cinchonidine 28·2 per cent., which, bearing in mind how considerably the rotation figures are influenced by the presence of hydroquinine or of even minute quantities of cinchonine ($[\alpha]_D$ = +224·4° Rabe), are in fair agreement with those arrived at from the methoxyl determination.

Crop B, similarly converted to base, gave for methoxyl 5·09 per cent. corresponding to quinine 53·2 and cinchonidine 46·8 per cent. The specific rotation ($c$ = 5 in alcohol) was −133·6° corresponding to quinine 47·9 and cinchonidine 52·7 per cent.

Crop C, consisting of cinchonidine tartrate, was also converted to base and examined in the same way. It gave 1·45 per cent. of methoxyl corresponding to 84·2 per cent. of cinchonidine and 15·8 per cent. of quinine. The specific rotation ($c$ = 5 in alcohol) was −112·8°, which is equivalent to 96·2 per cent. of cinchonidine and 3·8 per cent. of quinine. The discrepancy between the results obtained by

the methoxyl and the optical methods was so considerable in this case that the liquors from which the cinchonidine tartrate had crystallised were made alkaline and extracted with ether. From this ethereal extract 0·5 Gm. of colourless crystalline alkaloid was obtained, which was undoubtedly cinchonine, since it melted at 243°, and this melting point was raised to 247° by admixture with pure cinchonine, m.p. 251°. The presence of 1·56 per cent. of cinchonine in the cinchonidine tartrate would lower the specific rotation sufficiently to account for the discrepancy between the results of the methoxyl and optical determinations referred to above.

Calculating from the results of the three methoxyl determinations, the relative percentage proportions of the two alkaloids, quinine and cinchonidine in Bark D, are as follows :—

Quinine, 60·05 per cent. or 3·07 per cent. expressed on the bark.

Cinchonidine, 39·95 per cent. or 2·05 per cent. expressed on the bark.

These figures leave out of account 18·3 Gms. of alkaloidal sulphate and 7·3 Gms. of alkaloidal tartrate left in the recrystallisation liquors. These corrected results are a little higher in quinine than those commonly found for the relative proportions of quinine and cinchonidine in normal *Cinchona succirubra* bark, but sample D differs mainly from bark of this species in its high yield of total alkaloid. It probably represents bark from a tree in which the *C. succirubra* stock has become predominant over the *C. Ledgeriana* graft. Such cases are known to occur (Gage : *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1925, Vol. 18, p. 369). The abnormal *C. succirubra* bark referred to by Prof. Greenish ('Year-Book of Pharmacy,' 1923, p. 655) is probably another example of the same kind.

It is, of course, well known that the quinine sulphate obtained in Howard and Chick's method of quinine assay may, as the authors themselves state (Allen's 'Organic Analysis,' 4th Edition, Vol. 6, p. 491), contain other alkaloids than quinine, notably cinchonidine, and it seems likely that methoxyl determinations controlled by polarimetric readings may be useful in ascertaining the extent of the contamination by cinchonidine. The method does not discriminate between quinine and hydroquinine, but as evidence appears to be accumulating steadily that hydroquinine is of greater anti-malarial value than quinine and the amount of hydroquinine present is usually small, the

return of hydroquinine as quinine is not of great practical importance.

### Cinchona Febrifuge.

Attention has been directed in recent years to the great variability in the composition of "cinchona febrifuge," particularly by Howard and Chick ('Year-Book of Pharmacy,' 1923, p. 639), Howard (*Trans. Roy. Soc. Trop. Med. and Hygiene*, 1925, Vol. 8, p. 358), and Hooper (*Quart. Journ. Pharm. and Pharmacol.*, 1929, Vol. II., p. 186). It appears to us that the variability may be in part due to the fact that the processes available for the assay of such mixtures of cinchona alkaloids have been developed mainly for use with the total alkaloids of Java cinchona bark, and are not really suitable for cinchona febrifuge. Howard and Chick's process, for example, is essentially a process of fractional crystallisation of three salts used in series, and everyone who has had much experience of the fractionation of mixtures of alkaloidal salts containing amorphous material is aware how profoundly the yield and composition of the crystalline fractions obtained is influenced by the amount of amorphous material present. It is hardly to be expected, for example, that two mixtures of the following composition,

| | I. | II. |
|---|---|---|
| Quinine | 71·5 | 10·5 |
| Cinchonidine | 6·2 | 7·0 |
| Quinidine | 7·6 | 16·0 |
| Cinchonine | 4·3 | 23·0 |
| Amorphous alkaloids | 10·4 | 33·0 |

would on treatment by Howard and Chick's process yield fractions of "quinine sulphate," "cinchonidine tartrate," and "quinidine hydriodide" of the same composition. Column I. gives the average percentage composition of the total alkaloids of *Cinchona Ledgeriana* bark as cultivated in Bengal, and Column II. that of Indian cinchona febrifuge in 1922, and both are quoted by Col. A. T. Gage (*Trans. Roy. Soc. Trop. Med. and Hygiene*, 1925, Vol. 18, pp. 349, 350). It seems to us that it would be interesting to examine the fractions obtained in assays of typical febrifuges by the methods we have applied in the case of the total alkaloids of the Tanganyika bark D to see whether variation does occur and if so to what extent.

(*From the Wellcome Chemical Research Laboratories.*)

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