7. The blue value of amylose increases with chain length, approaching an asymptotic value. Conversely, it decreases on hydrolysis.

8. A hypothesis is suggested to account for the observed kinetics.

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The Distribution and Interrelationships of the Alkaloids in the Bark of *Cinchona Ledgeriana*

By H. F. BIRCH AND L. R. DOUGHTY

Cinchona Research Organization, Amani, Tanganyika Territory

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The literature on the interrelationships between the principal alkaloids and their distribution throughout the bark of trees of the cinchona species is scattered and rather sparse. The references available indicate that in general the alkaloid content is greater in the root bark and at the base of the tree than in the upper parts. Hodge (1946) found, however, that the reverse condition obtains in some wild Peruvian cinchonas. Sando (1944), who studied alkaloidal distribution in *Cinchona succirubra*, found a positive correlation between alkaloid content and tree girth.

The four main crystallizable alkaloids in the bark are the two groups of stereo-isomerides quinine and quinidine, and cinchonidine and cinchonine. Quinine and quinidine contain a methoxyl group in the 6-position of the quinoline nucleus, otherwise the molecular structures are the same. Such similarity in structure suggests a common mechanism of formation. The proportions in which the alkaloids are laid down in the bark, the wide differences in the proportions of the alkaloids according to bark species, and the smaller differences found among barks of the same species, and in the bark from various parts of a single tree are probably due to the operation of factors affecting the rates of conversion of one alkaloid to another.

The present investigations were undertaken to determine the distribution of the alkaloids (total,

individual and amorphous) throughout the whole bark, and to relate this distribution with the form of the tree, the metric characteristics of the bark and the proportions of the various alkaloids. Some interesting relationships have been established.

EXPERIMENTAL

Materials

The trees selected were of the Ledger species, approximately 7 years old and grown on the same plantation. Bark from *C. Ledgeriana* is the richest in quinine of all cinchona barks, and contains usually 8–12% of alkaloids, of which 3–8% is quinine.

Tree 2137 was felled at ground level and the trunk sawn into 25 cm. sections over a length of 4 m. from the base. The main stem was divided into two, 2 m. from the base. The barks from sections equidistant from the base along each of these stems were combined. The bark from each section was air-dried, weighed and ground to pass a 50-mesh sieve. The root bark was collected as one sample. The sections were numbered consecutively from the base upwards.

Tree 1970. The preliminary investigations on tree 2137 showed that the alkaloid distribution is to some extent a function of the form of the tree. A detailed drawing was therefore made of tree 1970 showing the location of secondary leaders (erect growing stems as opposed to more or less horizontal branches) as well as of living, dead and, as far as possible, former branches. The tree was of irregular girth with maximum trunk thickness about 130 cm. above ground, where there was also a marked bending of the trunk from the vertical for a distance of about 20 cm. (Fig. 1).

The main stem and the secondary leaders were cut into 10 cm. sections. The lateral branches were divided into four groups (primaries, secondaries, tertiaries and quaternaries) and the leaves into seven groups ranging from very young to mature. In the lower parts of the tree one section was selected at random from each 50 cm. length, except where the formation of the tree (as revealed by the preliminary

Analytical methods

The samples were analyzed for total alkaloids (TA), quinine (Q), cinchonidine (Cd), cinchonine (C), and amorphous alkaloids (Am), and the results expressed as a percentage of the moisture-free bark. Quinidine was present in traces only and was not quantitatively determined.

Fig. 1. Tree 1970. Diagram showing the form of the tree with the locations of the secondary leaders and the sections analyzed. The corresponding analytical data/10 cm. section are plotted on the left. ● - - - - ● Bark weight; ○ - - - - ○ percentage total alkaloids; ○ · · · · · ○ Q : Cd ratio. Average values of bark weight/10 cm. section are given for each of the four groups of composite samples above section 35.

work on tree 2137) was of special interest, e.g. where the main stem bent, or where big lateral branches arose. At such positions two or three contiguous sections were taken. Where the amount of bark from a 10 cm. section was insufficient for analysis, the bark from a sequence of five sections was bulked, i.e. a 50 cm. length of stem. For the lateral branch bark and leaves a composite sample was taken from each of the groups into which they had been divided. Prior to preparing the samples for analysis, the thickness, area, volume and weight (air-dry) of the bark from each section were measured; also the girth of each section and the corresponding areas of the trunk.

Tree 1512 was selected, in contrast to tree 1970, for its straight form and apparently normal growth (Fig. 2). It had produced one secondary leader only. A detailed drawing was made of the tree and the same sampling technique employed as that for tree 1970.

Fig. 2. Tree 1512. Diagram showing the form of the tree with the locations of the secondary leader and the sections analyzed. The corresponding analytical data/10 cm. section are on the left. ● - - - - ● Bark weight; ○ - - - - ○ percentage total alkaloids; ○ · · · · · ○ Q : Cd ratio.

Total alkaloids. Air-dry bark (20 g.) was mixed with CaO (12 g.), water (15 ml.) and 5N-NaOH (2 ml.). After standing overnight the mixture was extracted with benzene in a Soxhlet apparatus. The alkaloids were recovered from the benzene extract with 5% (w/v) H₂SO₄; the latter was then made alkaline and the liberated alkaloids extracted with chloroform. The bulk of the chloroform was distilled, and the residual extract washed with chloroform into a small tared beaker. After the addition of a small quantity of ethanol the solution was evaporated and dried to constant weight at 100°.

The individual alkaloids in the total alkaloids were determined by the *British Pharmacopoeia* (1932) method for totaquina.

RESULTS

The analytical data not shown in the figures are given in Table 1.

Table 1. *Analytical data for trees 2137, 1970, 1512*

Tree no.	Bark section no.	Alkaloids (% dry bark)			
		Quinine	Cinchonidine	Cinchonine	Amorphous alkaloids
2137	1	6.75	1.20	0.70	1.11
	2	7.44	1.19	0.65	1.09
	3	6.99	1.11	0.70	1.00
	4	7.48	1.41	0.96	1.08
	5	7.32	1.30	0.80	0.82
	6	6.67	1.06	0.74	1.38
	7	6.82	1.22	0.90	1.17
	8	5.87	1.93	1.47	1.25
	9	6.51	1.47	0.90	0.86
	10	6.21	1.35	0.93	0.96
	11	5.31	1.38	0.78	0.90
	12	5.99	0.85	0.62	1.01
	13	6.21	1.42	0.89	1.03
	14	6.01	1.19	0.83	0.85
	15	5.06	0.62	0.71	1.04
	16	5.14	0.97	0.67	0.99
1970	Root bark	5.68	1.60	1.81	1.22
	3	4.86	0.80	Trace	1.16
	7	4.58	0.92	Trace	0.97
	12	4.64	1.47	0.22	0.97
	13	4.77	1.09	0.31	0.88
	14	4.84	1.26	0.29	0.86
	15	4.23	0.94	0.11	1.30
	20	3.18	0.67	0.09	1.78
	24	4.66	1.11	0.19	0.82
	27	2.91	0.58	0.09	1.75
	30	3.07	0.59	0.11	1.92
	31	2.94	0.53	0.13	1.86
	35-39	2.86	0.56	0.10	1.00
	40-44	2.41	0.59	0.08	1.87
	45-49	1.82	0.26	0.02	1.39
	50-60	1.79	0.28	0.03	0.96
1512	2	5.38	0.71	Trace	0.91
	9	4.79	0.63	Trace	1.26
	14	3.77	0.50	Trace	1.19
	18	3.07	0.46	Trace	1.43
	23	2.61	0.42	Trace	2.04
	25	1.62	0.27	Trace	2.31
	26	3.50	0.69	Trace	1.90
	32	3.07	0.70	Trace	1.54
	33	2.63	0.63	Trace	2.08
	34	2.56	0.45	Trace	1.38
	35	—	—	—	—
	37	2.69	0.53	Trace	1.04
	40-44	2.40	0.43	Trace	1.43
	45-49	1.34	0.39	Trace	1.30
	50-54	1.21	0.27	Trace	1.80
	55-64	0.64	0.15	Trace	0.97

Tree 2137. The distribution of the total alkaloids in the bark is shown in Fig. 3. There was an overall but irregular decrease of total alkaloid content from the base of the tree upwards. The maximum alkaloid content was found 2 m. from the base (section 8) where the tree forked, with secondary maxima (sections of high alkaloid content relative to adjacent sections) at sections 4 and 13. Unfortunately, nothing had been recorded of the form of this tree at these levels.

Fig. 3 shows also a positive correlation between percentage total alkaloids and bark weight/section.

The regression equations (Table 2) show that the percentage of total alkaloids, as well as the percentages of quinine and cinchonidine, were positively and significantly related to bark weight. The regression of cinchonine on bark weight was possibly significant, and that of amorphous alkaloids not significant.

These equations are more fully discussed later, but it should be noted here that increases in bark weight were associated with significant increases in the percentages of each of the crystallizable alkaloids. The equations give, however, only a general picture of

the association between increasing bark weight and increasing alkaloid content. When the molecular ratios quinine to cinchonidine ($Q:Cd$) and quinine to cinchonine ($Q:C$) were calculated for each section, an inverse relationship was found between these ratios and bark weight/section (Fig. 3).

Fig. 3. Tree 2137. The relationships between bark weight, percentage total alkaloids, the quinine to cinchonidine ($Q:Cd$) and quinine to cinchonine ($Q:C$) ratio/25 cm. section of bark. The $Q:C$ ratios shown are half the true values. ●---● Bark weight; ○---○ percentage total alkaloids; ○---○ $Q:Cd$ ratio; ●---● $Q:C$ ratio.

There was a marked similarity in the alkaloid contents of the root bark, and the bark where the tree forked. In these samples the percentages of cinchonidine and cinchonine were much higher, and the $Q:Cd$, and $Q:C$ ratios much lower than in samples from any other part of the tree. The quinine contents at the two locations were also similar. Quinine content was at a maximum in the bark from the lower stem roughly at about 1 m. above the base. The amorphous alkaloid content showed little variation throughout the length of the tree.

Tree 1970. The form of this tree is shown in Fig. 1 with the distribution of the total alkaloids, the weight of bark from the various sections and the $Q:Cd$ ratios. As with tree 2137 the decrease of alkaloid content from the base of the tree up was

irregular, and wide fluctuations were associated with certain parts of the tree, in this case notably where the trunk bent and where the lower secondary leader arose. Zones of low alkaloid content were found at those parts of the trunk where there was no indication that branches were ever formed.

The regression equations (Table 2) show that the percentage of total alkaloids was positively and significantly related to the weight, thickness and volume of the bark. The percentages of quinine and cinchonidine, and possibly that of cinchonine, were likewise related to bark weight and thickness, while a negative regression, possibly significant, was found for the amorphous alkaloids. When the regressions of the percentage individual alkaloids/section on percentage total alkaloids/section were calculated, significant positive regressions were found for the three crystallizable alkaloids, and a not significant negative regression for the amorphous alkaloids (Table 2).

A further analysis of the data showed significant negative regressions for amorphous alkaloids on quinine and on cinchonidine, and very significant positive regressions for cinchonidine on quinine and cinchonine on cinchonidine (Table 3). When the individual alkaloids were expressed as percentage of the total alkaloids, the two negative regressions of amorphous alkaloids on quinine and on cinchonidine were very significant (Table 3).

With this tree, as with tree 2137, the $Q:Cd$ and $Q:C$ ratios were inversely related to bark weight or thickness (Fig. 1). A well-marked inverse relationship between bark weight and the $Cd:C$ ratios was also noted, an observation not made with tree 2137, throughout the length of which the $Cd:C$ ratios in the bark were fairly constant. The highest percentages of cinchonidine and cinchonine, and the lowest $Q:Cd$ and $Q:C$ ratios, were found where the tree bent and where the lower secondary leader arose. At the same places the quinine to amorphous alkaloid ratios were highest. The greatest percentages of quinine were also found here and at the base of the tree.

In the lateral branches there was a rapid fall in the alkaloid content of the bark, from the primaries to the tertiaries from 2.19 to 0.46% for total alkaloids, from 0.68 to 0.11% for quinine, and from 0.29 to 0.09% for amorphous alkaloids. Large-scale extractions of the leaves, separated into groups ranging from young to mature, showed total alkaloid contents varying between 0.055 and 0.223%. No correlation was found between age of the leaf and total alkaloid content, and no crystallizable alkaloids could be isolated.

Tree 1512. The shape of this tree, the distribution of the total alkaloids, the related bark weights/section, and the corresponding $Q:Cd$ ratios are shown in Fig. 2.

Taken in conjunction, Figs. 1 and 2 show how total alkaloid distribution is related to the form of the tree. In the lower half of tree 1512 the decrease in alkaloid content from the base upwards was, with relatively minor fluctuations, a gradual one. The first evidence of any living branches was at section 32, and between this and section 34, where there were no branches, the alkaloid content fell rather rapidly, but rose again at section 35 where the only secondary leader arose. Thereafter the alkaloid content decreased, but with two secondary maxima for the bulk samples taken from sections 40-44 and 50-54. These two sections had, on an average, a larger number of lateral branches than sections 45-49.

The regressions of percentage total alkaloids/section on bark weight, thickness and volume/section (Table 2) were of the same high order of significance as those for tree 1970. The interrelationships between bark weight, tree form and total alkaloid distribution appear therefore to be fairly well established.

Increases in bark weight were also associated with significant increases in the percentages of quinine and cinchonidine, but the increase in amorphous alkaloids was not significant (Table 2). Increases in percentage quinine were accompanied by significant increases in cinchonine, but no significant relationship was found between these two alkaloids and the amorphous alkaloids (Table 3). When, however, the alkaloids were expressed as a percentage of the total alkaloids a highly significant negative regression was found for amorphous alkaloids on quinine, and a possibly significant negative regression for amorphous alkaloids on cinchonidine (Table 3).

In contrast to trees 1970 and 2137 the bark from tree 1512 contained insufficient cinchonine for a quantitative determination. Moreover, the $Q : Cd$ ratios of the samples taken from the lower half of the tree decreased from the base of the tree upwards. At sections 26, 32 and 33, where the $Q : Cd$ ratios were relatively low, there were, respectively, two dead branches, two living branches and a dead and

Table 2. *Regressions of the total and individual alkaloids on bark weight, thickness and volume, and of the individual alkaloids on total alkaloids*

(Y_{TA} , Y_Q , etc. are the calculated percentages of total alkaloids, quinine, etc. per section for any value of X (bark wt., g.), X_1 (bark thickness, mm.), X_2 (bark volume, ml.) or X_3 (percentage total alkaloids), per section, lying within the range of values found for the sections analyzed. P shows the level of significance of the regression. N.S. not significant.)

	Tree no. 2137 (25 cm. section)	P	Tree no. 1970 (10 cm. section)	P	Tree no. 1512 (10 cm. section)	P
Y_{TA}	$7.14 + 0.0223 X$	0.01	$4.220 + 0.0565 X$	0.01	$3.47 + 0.084 X$	0.01
Y_Q	$4.45 + 0.0114 X$	0.01	$1.645 + 0.0523 X$	0.01	$0.371 + 0.077 X$	0.01
Y_{Cd}	$0.42 + 0.0048 X$	0.01	$0.210 + 0.0154 X$	0.01	$0.256 + 0.007 X$	0.01
Y_C	$0.45 + 0.0022 X$	0.10	$0.031 + 0.0024 X$	0.10	—	—
Y_{Am}	$0.90 + 0.0008 X$	N.S.	$1.654 - 0.0096 X$	0.10	$1.453 + 0.002 X$	N.S.
Y_{TA}	—	—	$0.88 + 1.0885 X_1$	0.01	$0.40 + 1.320 X_1$	0.01
Y_{TA}	—	—	$4.25 + 0.0162 X_2$	0.01	$3.16 + 0.036 X_2$	0.01
Y_Q	—	—	$-1.870 + 0.864 X_3$	0.01	—	—
Y_{Cd}	—	—	$-0.814 + 0.253 X_3$	0.01	—	—
Y_C	—	—	$-0.170 + 0.046 X_3$	0.05	—	—
Y_{Am}	—	—	$-1.914 - 0.098 X_3$	N.S.	—	—

Table 3. *Regressions of the individual alkaloids per section. A for the alkaloids expressed as percentage of the bark, B for the alkaloids expressed as percentage of the total alkaloids*

(Y_{Am} , Y_{Cd} , etc. are the calculated values for percentage of amorphous alkaloids, cinchonidine, etc. per bark for any value of quinine (X_Q), cinchonidine (X_{Cd}) or cinchonine (X_C) percentage bark lying within the range of values found for the sections analyzed. Similarly with Y_{Am}' , X_Q' , etc., but these are expressed as a percentage of the total alkaloids. P shows the level of significance of the regression. N.S. not significant.)

A	Tree no. 1970	P	Tree no. 1512	P
Y_{Am}	$2.006 - 0.198 X_Q$	0.05	$1.754 - 0.091 X_Q$	N.S.
Y_{Am}	$1.796 - 0.640 X_{Cd}$	0.05	$1.602 - 0.206 X_{Cd}$	N.S.
Y_{Am}	$1.466 - 1.419 X_C$	0.30	—	—
Y_{Cd}	$-0.216 + 0.278 X_Q$	0.01	$0.183 + 0.109 X_Q$	0.01
Y_{Cd}	$-0.040 + 0.044 X_{Cd}$	0.10	—	—
Y_C	$-0.034 + 0.196 X_{Cd}$	0.01	—	—
B				
Y_{Am}'	$71.11 - 0.881 X_Q'$	0.01	$48.57 - 0.533 X_Q'$	0.01
Y_{Am}'	$44.48 - 1.910 X_{Cd}'$	0.01	$39.29 - 1.750 X_{Cd}'$	0.10
Y_{Am}'	$27.15 - 2.820 X_C'$	0.10	—	—
Y_{Cd}'	$-4.25 + 0.287 X_Q'$	0.01	$4.023 + 0.088 X_Q'$	0.02
Y_{Cd}'	$-0.34 + 0.038 X_{Cd}'$	0.40	—	—
Y_C'	$-0.82 + 0.222 X_{Cd}'$	0.02	—	—

a living branch when the tree was felled. At section 34, where the $Q : Cd$ ratio was relatively high, there were no branches. In the upper half of the tree, where many living branches joined the main stem, there was a marked fluctuation in the $Q : Cd$ ratios. The highest quinine contents were found in the three sections taken from the lowest quarter of the tree, roughly between ground level and 1.5 m.

In the lateral branches there was a rapid fall in the total alkaloid content of the bark from the primaries to the quaternaries from 2.71 to 0.68 %. Large-scale extractions of the leaves separated into groups ranging from young to mature showed total alkaloid contents ranging from 0.16 to 0.33 %, but no correlation was found between leaf age and total alkaloid content. The combined total alkaloids (1 g.) from the leaves gave a tartrate equivalent to 0.0156 % quinine plus cinchonidine in the leaves, but neither quinine nor cinchonidine could be isolated from the decomposed tartrate.

DISCUSSION

The distribution of the alkaloids in the stem bark is a function of bark thickness. In general the thickness of the bark at any point is inversely proportional to the distance of that point from the base of the tree, but a regular decrease of bark thickness from the base upwards is rare. In these investigations local thickening of the bark was found where the main stem forked, usually caused by damage of the stem at these points at an earlier stage in the tree's history, where secondary leaders arose, probably due to a similar cause, and where large branches joined the stem. Superimposed, therefore, on what is intrinsically a regular decrease in alkaloid content from the base of the tree upwards, are zones of relatively high alkaloid content, the locations of which are governed in each tree by the history of its growth as reflected in its form.

Table 2 shows, for the stem bark as a whole, the increases or decreases in alkaloid content that had been associated with unit increase in bark weight during the life of the tree. Significant increases were found for total alkaloids, quinine and cinchonidine/unit increase in bark weight, and for quinine cinchonidine and cinchonine/unit increase in total alkaloids. These increases were large compared with the small and insignificant increases (trees 2137 and 1512) and decreases (tree 1970) found for the amorphous alkaloids, an indication of the progressive conversion of amorphous to crystallizable alkaloids during the life of the tree. When the trees were felled the amorphous alkaloid content was that currently available for transformation to the crystallizable alkaloids, while the crystallizable alkaloid content represented the accumulation of the end products of the amorphous alkaloid transformations throughout tree growth.

The negative regressions shown in Table 3 lend support to the theory that the amorphous alkaloids are the precursors of the crystallizable alkaloids, additional evidence for which is furnished by observations made here that the bark of young trees, or from the younger upper parts of an old tree, contain a high proportion of amorphous in relation to crystallizable alkaloids.

The proportions in which quinine, cinchonidine and cinchonine are laid down must depend on the conditions obtaining in the bark when the amorphous alkaloids are converted. If the crystallizable alkaloids were formed simultaneously and in definite proportions, their ratios would be constant throughout the bark irrespective of its position on the tree. It was found, however, that the $Q : Cd$ and $Q : C$ ratios were notably low in the root bark and in the bark where the main stem forked, where secondary leaders arose, and where there was abnormal bark thickening. These low ratios do not imply a low quinine content, but rather a relatively greater increase of cinchonidine and cinchonine compared with that of quinine. It is evident that there is a preferential formation of cinchonidine and cinchonine where there is increased cambial activity following some form of injury or other stimulus, further evidence for which is furnished by the almost complete lack of cinchonine in the normally growing tree 1512. These observations, in conjunction with the relationships already established between bark thickness, total alkaloid content and abnormal bark growth, indicate that cambial activity plays a major role in determining the distribution of the total alkaloids in the bark, and the proportions in which the crystallizable alkaloids are laid down. Cambial activity may further govern the efficiency or degree of conversion of the amorphous to the crystallizable alkaloids, for in tree 1970 the highest crystallizable to amorphous alkaloid ratios obtained where the tree bent and where the secondary leader arose.

Botanical investigations in Java (private communication) have shown that a high quinine content is found where vertical growth is stunted and that in such areas *helopeltis* attacks are common, which further restrict growth and increase the quinine content. While a valid comparison of the average percentages of the individual alkaloids in the three trees here investigated is not strictly admissible (since the bark samples were not selected purely at random), it appears from the analytical data that tree damage was associated with an increased percentage of crystallizable alkaloids and a decreased percentage of amorphous alkaloids as compared with those in the normal tree, a further indication of some relationship between cambial activity and the transformation of amorphous to crystallizable alkaloid.

The high percentages of total alkaloids associated with local bark thickening suggests an increased translocation of amorphous alkaloids or their precursors to these places, possibly as soluble salts. The low $Q : Cd$ and $Q : C$ ratios coincident with abnormal thickening, and the variation in the proportions of the individual alkaloids and their ratios from bark section to bark section, indicate that the crystallizable alkaloids, once formed, are not further translocated. Their movement seems to be restricted towards the outer periphery of the bark as it ages and thickens, while further transformations of amorphous to crystallizable alkaloid take place in the newer underlying bark. It is unlikely that the proportions in which the alkaloids are laid down in any particular section of bark remain constant during tree growth. The association between decreasing $Q : Cd$ ratio and increasing height of bark above ground level found in the lower half of the normal tree suggests that, at least under normal conditions of growth, the transformation of amorphous to crystallizable alkaloids shifts progressively in favour of quinine as the bark ages, possibly as a result of a progressive change in cambial activity.

Dawson (1945), in his work on the physiological chemistry of the tobacco alkaloids, has demonstrated that normicotine is produced *in vivo* from nicotine. From a consideration of their respective molecular structures he suggested that nicotine is acted upon in the cell by a transmethylating agent and a methyl acceptor (RH) and proposed as a working basis the hypothetical mechanism

This faculty, he considered, is inheritable.

The simultaneous increase of quinine, cinchonidine and cinchonine in the bark does not preclude the possibility that quinine may at the same time be degraded to cinchonidine and cinchonine, but at a slower rate than its synthesis from the amorphous alkaloids. The transformation may be postulated as

This reaction assumes the presence of a trans-methoxylating agent and a methoxyl acceptor, the reaction rate depending on the RH content of the bark, which is primarily governed by the age of the bark and its location on the tree. Such a mechanism could account for the wide fluctuations observed in the $Q : Cd$ and $Q : C$ ratios in the bark of any particular tree and, if inheritable, for the predominance of quinine in *C. Ledgeriana* and cinchonidine in *C. succirubra*.

SUMMARY

1. Alkaloid distribution in the bark of three trees of *Cinchona Ledgeriana* has been investigated. Two of the trees had suffered damage at an earlier stage of their growth and differed markedly in shape from the third tree which was of apparently normal growth and straight in form.

2. Significant positive regressions were found for percentage total alkaloids on bark weight, thickness and volume. Increases in bark weight and total alkaloids were associated with significant increases in quinine, cinchonidine and cinchonine at the expense of the amorphous alkaloids.

3. Local thickening of the bark was found where the tree forked, where secondary leaders arose and where the tree had been damaged. At these places the alkaloid content was notably high and the quinine : cinchonidine ($Q : Cd$) and quinine : cinchonine ($Q : C$) ratios relatively low. Similar conditions obtained in the root bark. Local thickening was less pronounced with the normal tree, the bark of which showed, from the ground upwards, a fairly regular decrease in the percentage of total alkaloids and in the $Q : Cd$ ratios, in contrast to the wide fluctuations observed with the other trees.

4. Meristematic or cambial activity appears to be an important factor in determining the alkaloid content of the bark, the degree of conversion of the amorphous to the crystallizable alkaloids, and the proportions in which the latter are laid down. The distribution of the alkaloids throughout the bark is therefore primarily governed by the history of the tree as reflected in its form.

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