6. Exposure of amylose to hot water brings about changes even in the portion which does not retrograde. The blue value falls and β -amylase action is incomplete.

7. Amylopectin was not so successfully purified, but fractional precipitation with ethanol freed it

slightly from amylose which had escaped the first precipitation.

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The Action of β -Amylase on Potato Amylose

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It has long been known that almost all natural starches contain a constituent which can be hydrolyzed to completion by β -amylase. It has been given various names, that of amylose being the one used here. It is only in quite recent years that amylose has been prepared in a state of purity (Schoch, 1942; Haworth, Peat & Sagrott, 1946). β -Amylase has usually been reported as promoting the hydrolysis of amylose to 90–100% of maltose, but many of these figures may be above the truth. Often, it has not been made clear whether the figure reported referred to maltose expressed as a percentage of the moisture-free starch used or of the maltose theoretically obtainable from it. The absence of glucose from the products has been assumed, although many preparations of β -amylase contain maltase. Lastly, it is rare that the β -amylase has been demonstrated to be free from α -amylase and from maltase.

In this work, the purest amylose and β -amylases obtainable have been investigated with special attention to the kinetics of the reaction and a hypothesis is advanced to account for the facts.

MATERIALS AND METHODS

Substrates. The various preparations of amylose are described elsewhere (Hopkins & Jelinek, 1948). Solutions not exceeding 0.9% concentration were prepared by heating the butanol-amylose complex in water until the butanol was driven off.

Blue value (B.V.). The procedure of McCready & Hassid (1943) as modified by Hopkins & Jelinek (1948) was employed.

Red value (R.V.). This was measured in the same way as the B.V., except that filters 602 and 603 were used with the

Spekker absorptiometer. It was found of advantage, however, to increase the iodine concentration from 0.002 to 0.008%.

Enzyme preparations. β -Amylase was prepared (1) from well-ripened barley as described by Hopkins, Murray & Lockwood (1946), (2) from soya beans by the method of Newton & Naylor (1939) and Newton, Hixon & Naylor (1943). Both specimens were tested and found to be free from maltase activity under the appropriate conditions of use. The limits of hydrolysis of starch pastes were 57% of the theoretical maltose ($R_m = 57\%$), and the preparations were considered to be free from α -amylase.

Reaction mixtures. These were as employed by Hopkins *et al.* (1946), but contained varying concentrations of amylose as stated in the tables, and were buffered at pH 4.6 for barley and pH 5.8 for soya amylase. Progress of hydrolysis at 25° was determined as described by Hopkins *et al.* (1946), except that the hypoiodite titration was found not to be wholly satisfactory in the presence of certain antiseptics or if a trace of butanol was present. In such cases, the method of Cole (1933) was substituted, the ferricyanide being calibrated against maltose. Withdrawals from the reaction mixture were made into boiling water.

Calculation of the values of ' k ' as if the reaction were monomolecular revealed that these decline progressively and markedly through the reaction. As is shown later, there was no theoretical reason for expecting monomolecularity.

For the determination of the affinity constant, a series of flasks was set up containing varying concentrations of the amylose (about four flasks for each concentration). All flasks contained identical concentrations of enzyme and buffer. After suitable intervals of time (estimated from a pilot experiment) pH 10 buffer was added to the respective flasks to check the reaction and the maltose determined by titration. The velocity of reaction v (mg. maltose/100 ml./min.) was obtained for each concentration of amylose substrate from the initial slope of the progress curves.

RESULTS

The limit of action was usually 99 % R_m , the full 100 % being obtained in some cases. An example is given in Table 1. The final reaction mixture from this experiment was concentrated after distilling off traces of antiseptics. The reducing power in a sample was determined by hypiodite titration. After making allowance for weight and reducing power of the enzyme, the results indicated pure maltose. $[\alpha]_D^{25}$ was found to be +136°.

Table 1. Action of β -amylase (soya) on potato amylose (B.V. 0.332)

(Concentration of amylose in reaction mixture 0.41%)			
Reaction time (hr.)	% of theoretically obtainable maltose (R_m)	Reaction time (hr.)	% of theoretically obtainable maltose (R_m)
0.5	49.3	3.0	85.4
1.00	62.7	3.5	88.4
1.33	68.5	4.0	89.3
1.5	71.0	5.0	92.8
1.75	74.1	20	97.6
2.0	76.4	90	99.1
2.5	82.2	192	100.0

Amylose prepared from autoclaved starch paste (120° for 2 hr. at pH 7, Table 2, no. 1) underwent hydrolysis about 1.5 times as fast as that similarly obtained from paste prepared at 90° (no. 2), but

each reached the same limit. It may be remarked that the amylopectins prepared at the same time underwent hydrolysis at substantially the same rate as one another.

The reaction of the starch paste at the time of crystallization of amylose influenced the rate at which the amylose subsequently became hydrolyzed at pH 5.8. Of the amyloses in Table 2, one series, nos. 3, 4 and 5, had been precipitated respectively at pH 4.1, 6.0 and 9.4 by butanol, whilst nos. 6 and 7 constituted a pair precipitated at pH 4.1 and 9.4 respectively by cyclohexanol followed by thymol. The choice of precipitant proved to be relatively unimportant, but the amyloses precipitated at acid reaction underwent hydrolysis the most rapidly. Associated with their liability to retrograde, discussed elsewhere (Hopkins & Jelinek, 1948), they failed to reach as advanced a limit of hydrolysis as the other samples. No. 3 partly came out of solution in hot water on two occasions before enzyme action was commenced. The substrate remaining in solution was hydrolyzed to an even lower limit (no. 8) than the whole sample (no. 3). During heating, some had precipitated, but more had been changed ('denatured') into a form less susceptible to β -amylase. The purer, i.e. the more 'native' the amylose, the more completely it is hydrolyzed and the higher its blue value (Fig. 1).

The results in Tables 1-3, e.g. nos. 1 and 2 of

Table 2. Action of β -amylase (soya) on potato amyloses

(Only reaction mixtures of a given series as mentioned in the text received identical quantities of the enzyme.)

(Results as percentage of theoretically obtainable maltose.)

No. of amylose sample (see text)	...	1	2	3	4	5	6	7	8	9
Feature of its preparation	...	Paste heated at 120°	Paste heated at 90°	Pre-cipitated at pH 4.1	Pre-cipitated at pH 6	Pre-cipitated at pH 9.4	Pre-cipitated at pH 4.1	Pre-cipitated at pH 9.4	No. 3 partly retro-graded	—
Blue value	...	0.322	0.332	0.285	0.310	0.342	0.308	0.326	0.252	0.327
% Amylose in reaction mixture	...	0.61	0.45	0.58	0.59	0.58	0.25	0.26	0.42	0.40
Time										
10 min.		25.0	19.5	19.9	14.8	12.8	20.1	13.3	26.0	—
20 min.		44.4	29.3	—	24.2	—	37.0	22.4	—	—
30 min.		—	—	—	—	—	—	—	47.4	—
40 min.		52.8	37.9	53.2	37.2	32.5	54.0	37.2	—	—
60 min.		61.7	46.7	—	46.4	40.1	63.9	46.0	—	—
90 min.		69.4	55.6	—	55.1	47.6	—	—	—	—
120 min.		73.8	—	—	60.3	54.2	81.6	62.3	—	—
150 min.		—	64.7	—	—	—	—	—	—	13.4
180 min.		79.6	66.7	—	69.5	62.6	86.0	69.3	—	—
210 min.		81.3	72.0	—	—	—	—	—	—	—
240 min.		82.4	74.0	—	—	—	87.3	75.1	—	—
300 min.		83.1	77.4	—	76.0	72.0	88.3	82.2	78.0	25.7
360 min.		84.4	80.4	—	—	—	—	—	—	29.6
435 min.		—	—	—	—	—	—	—	—	33.8
465 min.		—	—	—	—	—	—	—	—	34.9
11 hr.		—	—	—	—	—	—	—	—	44.2
24 hr.		—	—	91.5	91.5	95.9	92.5	95.0	82.5	62.2
Limit		97.4	98.0	94.0	—	98.0	94.8	99.0	85.5	—

Table 3, and the curves *A* and *B* of Fig. 1 show that the rate of hydrolysis is never constant but declines steadily. Except in the case of the partly 'denatured' amylose samples (e.g. nos. 3 and 6 of Table 2) there is no sudden break in the curve if percentage maltose is plotted against time. This

Fig. 1. Progress of hydrolysis of amylose by β -amylase (soya). The curves beyond 5 hr. were interpolated, making use of later readings which are not within the range of the graph. Curve *A*. From Table 2, no. 5. Limiting % 98. Curve *B*. From Table 1. Limiting % 100. Curve *C*. From Table 2, no. 6 (partly denatured amylose). Limiting % 95.

means that there is no evidence from our results of any branched or laminated chains in amylose, as has been suggested by other workers who observed

such breaks. These do occur, however, with partly 'denatured' amylose samples, and the hydrolysis does not reach the full 100% (Fig. 1, curve *C*).

Fig. 2. Initial velocity-substrate concentration relationship plotted according to the procedure of Lineweaver & Burk (1934) for the system β -amylase (soya)-amylose. For amylose preparations *A*-*D* inclusive, see text.

	<i>V</i> (max. velocity) (mg. maltose produced/100 ml./min.)	<i>K_m</i>
<i>A</i>	12.0	1.17
<i>B</i>	5.45	0.53
<i>C</i>	6.25	0.86
<i>D</i>	6.05	0.45

Returning to the steady decline in velocity, this is more pronounced than if the reaction were monomolecular. At the same time the β -amylase-amylose

Table 3. Action of β -amylase (barley) on potato amylose
(Results as percentage of theoretically obtainable maltose.)

No. of sample (see text)	...	1	2	3
Feature of its preparation	...	Precipitated by thymol (not autoclaved)	Precipitated by butanol (not autoclaved)	Mixed samples
Blue value	...	0.345	0.355	0.336
% Amylose in reaction mixture	...	0.72	0.72	0.63
Time				
15 min.		7.5	—	22.2
30 min.		12.2	11.9	32.6
45 min.		16.4	—	—
60 min.		20.1	20.0	45.6
90 min.		26.3	—	53.2
120 min.		31.7	34.0	58.9
180 min.		—	44.4	66.8
240 min.		52.4	53.8	72.2
300 min.		—	61.4	75.8
360 min.		—	68.5	79.2
420 min.		—	74.2	—
480 min.		—	77.7	—
24 hr.		97.1	—	94.8
Limit		99.5	100.0	100.0

system complies with the Michaelis & Menten formulations in certain respects, since on plotting reciprocals of velocity ($1/v$) against those of concentration ($1/x$) straight lines were obtained (Fig. 2). The values of K_m (the affinity constant) and V (the maximum velocity attainable) were deduced by the usual procedure (Lineweaver & Burk, 1934). It is interesting to note that the affinity constants varied with specimens of amylose according to the conditions of precipitation. The 'amylose A' (Fig. 2) was precipitated from potato paste prepared at 100° by the addition of cyclohexanol (0.15 %) and thymol (0.15 %) simultaneously at 25° following the sug-

Michaelis formula. This is associated probably with 'denaturation' of the amylose under such conditions.

When amylose is undergoing hydrolysis with β -amylase the molecular chains are being progressively shortened. By withdrawing a portion of the reaction mixture, stopping the hydrolysis in it and recovering the residual polysaccharide by the butanol precipitation method, specimens were obtained of amylose in such stages of degradation. These in turn, when set up in identical reaction mixtures, were found to be hydrolyzed at widely different rates; the less degraded the amylose, the faster it underwent hydrolysis (Table 4). The partly

Table 4. Action of β -amylase on amylose, previously partly degraded

(Results as percentage of theoretically obtainable maltose.)

No. of sample (see text)	...	Original	<i>E</i>	<i>F</i>	<i>G</i>
Blue value	...	0.327	0.335	0.327	0.339
% Substrate in reaction mixture	...	0.324	0.322	0.330	0.316
Time					
12 min.		24.0	—	20.6	15.6
22 min.		—	37.7	30.0	21.8
44 min.		58.5	51.6	44.4	31.7
60 min.		—	57.7	47.6	32.6
90 min.		—	62.0	—	—
150 min.		—	73.5	60.9	44.9
210 min.		—	79.2	69.6	51.0
300 min.		—	85.9	75.5	55.2
20 hr.		91.8	95.5	89.9	85.3

gestion of Haworth *et al.* (1946). Amylose separated in 3–5 days and was removed ('amylose A'). The residual paste was then heated to 100° and cooled slowly, more amylose separating ('amylose B'). Each amylose was purified as described by Hopkins & Jelinek (1948). The first fraction to precipitate (*A*) gave the higher value of K_m , but as K_m is expressed as a percentage and not as molarity (the mean molecular weight being unknown) this does not necessarily mean that this amylose possessed less affinity for β -amylase than the other. Evidence to be cited later indicates that the larger the amylose molecules the faster do they hydrolyze. This agrees with the high value of V for the first precipitate, the assumption being that it was the longer chain molecules which aggregated and came out of the paste the more readily.

Specimens *C* and *D* (Fig. 2) refer to two amylose 'fractions' prepared from one purified sample by partial saturation of its hot aqueous solution with butanol and slow cooling. Half of the amylose (*C*) separated from solution and was centrifuged off. The rest was recovered by full saturation (*D*). Again, the more precipitable fraction gave the higher value of K_m and of V . Evidently the numerical value of K_m for amylose and β -amylase varies widely according to conditions of preparation. It may bear a relationship to mean chain length. There are indications that in relatively concentrated solutions of amylose, v does not increase as predicted from the

hydrolyzed specimens were obtained by withdrawal from reaction mixture no. 9 of Table 2, *E* at 13.9 %, *F* at 44.2 % and *G* at 69 % (after 46 hr.). It seems clear that the further amylose is degraded by β -amylase, the more difficult it becomes for the amylase to hydrolyze it, assuming that no change in molecular character has occurred during the recovery of the partly hydrolyzed fractions.

For the experiments reported in Table 5 enzyme action was checked by withdrawing samples and running into alkali instead of boiling water, since boiling may change the blue value of amylose. The percentage hydrolysis was determined by hypoiodite, and, after calculation of the concentration of residual polysaccharide, the liquid was diluted and used for the measurement of blue and red values. It was ascertained that these values were not influenced by the presence of maltose or the enzyme preparation. Samples *H* and *K* were prepared from amylose in the same way as *B* and *A* respectively of Fig. 2. Sample *H* was a normal sample, whilst *K* had been very slightly 'denatured' as shown by its behaviour.

Filters 602 (4300–4700 Å. transmission) and 603 (4700–5100 Å.) may be regarded as transmitting light which will be absorbed by red liquids. Readings in the Spekker absorptiometer using these filters are therefore 'red values' and will be relatively high in the presence of amylopectin or of dextrans of chain length about 7–12 glucose units. On the other

hand, with filter 607 (5900–6300 Å.) scarcely any absorption by red solutions is measured, and with filter 608 none at all. With the latter filters, the absorption by amylose of greater mean chain length than 12 is measured. Only the values with filters 603 and 608 are recorded, but in several experiments a close parallel was observed between the 602 and 603 readings and also between the 607 and 608 readings.

expected since the formation of the reaction product, maltose, is not accompanied by a corresponding diminution of the molar concentration of the substrate. The percentage concentration of the latter diminishes, yet the molar concentration remains unaltered until one or more chains in the system become too short for further hydrolysis, either in the form of maltose or a trisaccharide. If all amylose molecules were attacked with equal

Table 5. *Changes in blue and red values of residual polysaccharide during hydrolysis*

(Hydrolysis in percentage of theoretically obtainable maltose.)

Sample	...	<i>H</i> (normal sample)				<i>K</i> (slightly 'denatured')			
% Amylose in reaction mixture	...	0.64				0.62			
Time (min.)		Hydrolysis	Blue value (filter 608)	Red value (filter 603)	Ratio 608/603	Hydrolysis	Blue value (filter 608)	Red value (filter 603)	Ratio 608/603
0	0		0.346	0.131	2.64	0	0.327	0.127	2.56
40	56.0		0.327	0.130	2.52	53.4	0.282	0.117	2.41
70	66.5		0.310	0.129	2.40	61.1	0.258	0.107	2.41
100	74.3		—	—	—	67.6	0.247	0.102	2.42
130	78.6		0.280	0.120	2.33	72.2	0.230	0.100	2.30
160	82.0		0.255	0.114	2.24	75.2	0.199	0.090	2.21
240	88.6		0.220	0.109	2.02	81.9	0.164	0.077	2.13
300	92.2		0.179	0.100	1.79	86.0	—	0.066	—
360	93.8		0.128	0.084	1.53	88.0	0.084	0.048	1.75
420	95.0		0.078	0.069	1.13	89.8	0.040	0.039	1.03
500	96.9		0.059	0.076	0.77	90.7	0.021	0.032	0.66

DISCUSSION

In explanation of the various observations reported above the following hypothesis is advanced. Amylose consists of a mixture of chain molecules of various lengths, i.e. of a homologous series, the majority, according to the law of distribution, being of intermediate length. All but the relatively very short ones possess approximately the same blue value. Fractional precipitation, both from starch paste and from purified amylose, brings out the longer chains first, the shorter needing more drastic conditions to pick them out from the amylopectin, or to aggregate and crystallize them from aqueous solution. Heating changes the coiled pattern of the native macromolecules and renders them less blue in presence of iodine, less soluble in water and less completely hydrolyzable by β -amylase. With prolonged heating irreversible retrogradation occurs.

The longer chains are attacked preferentially and the more rapidly by β -amylase. The action of β -amylase is in one respect different from most enzymic reactions in which a single substance is decomposed. Successive maltose molecules are formed by the removal of two glucose groups at a time from the non-reducing end of the chain, a molecule of polysaccharide and a molecule of maltose being formed in each individual reaction. The more usual kinetics of an enzyme reaction cannot be

readiness, irrespective of chain length, a zero-order reaction would be expected, whatever the concentrations of substrate and enzyme employed. This would persist until the shortest chains were exhausted and, unless the substrate was suitably dilute, until inhibition by reaction products or inactivation of the enzyme interfered. A zero order reaction, however, is never observed, not even with the highest concentrations of substrate employed.

On the other hand, if we assume that the longer the chain the faster it is hydrolyzed, we should expect a steadily declining rate of reaction, as is invariably observed. It could not be of zero order over any appreciable percentage of hydrolysis. But there is no reason to suppose that it would be monomolecular. With low concentrations of substrate and fast reactions the value of k should actually fall, as was usually observed. A consideration of the results in Table 4 will make this clear. Amylose *G* must have possessed a mean chain length of about one third of that of *E*, since it had previously been hydrolyzed by β -amylase to 69%, compared with 13% for *E*. As each was now freshly hydrolyzed at the same percentage concentration, the molarity of *G* in the reaction mixture must have been about three times that of *E*. At the concentration employed, 0.32%, the latter would largely control the rate of maltose formation, as may be deduced from the affinity constants. We should therefore expect *G* to be hydrolyzed at something like three times the

rate of E , but it was hydrolyzed at about half the rate. With decreasing chain length the rate of β -amylase action falls off more than proportionately.

It appears that β -amylase combines with the substrate, and as regards the initial velocity of the reaction it conforms with the Michaelis & Menten theory. Owing, however, to the non-homogeneous nature of the substrate, the subsequent time progress relationship is not that usually associated with hydrolytic enzyme actions which obey the Michaelis & Menten relationship. It is a case of several substrates competing for the same enzyme.

It is assumed that the affinity constant, K_m , varies according to the mean chain length of the amylose, and when expressed as molarity decreases with increase of chain length. But the effect of increase of chain length on the value of K_m , expressed as percentage, would be to increase it simultaneously in proportion to molecular weight.

To distinguish between samples of amylose of different mean chain length by β -amylase action, a low concentration of substrate is desirable so that the latter controls the reaction velocity. It was for this reason that such conditions were imposed when amyloses precipitated under different conditions, or previously hydrolyzed to various stages, were under comparison. In the latter case, samples F and G (Table 4), being partly hydrolyzed, must have had relatively low mean chain lengths, and therefore relatively high molar concentrations compared with E . Nevertheless, they were hydrolyzed much more slowly. This experiment, moreover, eliminated the complication of inhibition of enzyme action by maltose.

We may therefore conclude that β -amylase attacks more readily the longer chain molecules and, provided no denaturation has taken place, hydrolysis proceeds at a steadily declining rate with no sudden change, continuing until 100% maltose has been formed.

When amylose is hydrolyzed by α -amylase or acid, the iodine coloration passes from the blue through purple and red to colourless. We have results indicating that the colourless stage corresponds to dextrins composed of about 6 glucose units, and the red stage to 10 units or so. This is supported by certain observations on synthetic products reported by Cori, Swanson & Cori (1945). When, however, β -amylase is used some blue colour remains until the hydrolysis has advanced well beyond 90%.

The relationship between the blue value and mean chain length of amylose is indicated by the results in Table 5. Whilst blue value is zero for short chains, it becomes measurable at about chain length 12, and increases rapidly with chain length. Later it approaches an asymptotic value (cf. specific optical rotation). Conversely, as the chain length is reduced by β -amylolytic action, blue value declines but little during the first 70% hydrolysis, but later falls more rapidly, disappearing at about 95%. The red value is slightly

later in falling, otherwise its behaviour is similar. Most, if not all, of the red value of the original amylose is due to the same colouring matter as the blue value and is numerically proportional to it. The ratio 608:603 reading remains approximately constant for 75% of the hydrolysis, which would scarcely be expected if there were present in the original amylose a separate fraction such as short-chain material, giving a red colour with iodine. However, at the point where material giving blue with iodine disappears through hydrolysis, the blue value would rapidly decline, whilst the corresponding decrease in red value would be delayed. Indeed, the latter might in certain circumstances increase numerically for a time, depending on whether short-chain material giving a red iodine colour were formed fast enough to more than counterbalance the loss of red colour due to destruction of iodine-blueing polysaccharide. In at least one experiment a small rise in red value at about 80% was observed. However, owing to the possibility of appreciable error creeping in when computing the concentration of residual polysaccharide at late stages of the hydrolysis, neither the blue nor the red values in these late stages should be taken as accurate. Their ratios are significant, since the blue and red readings were taken on the same diluted material, and the above source of error is cancelled out. The ratio of 608:603 reading falls markedly after 80% hydrolysis, indicating the disappearance of amylose of more than 12 units chain length and generally supporting the conclusions drawn above. Apparently 80–90% or more of amylose is hydrolyzed before the mean chain length is as low as 10–12. Some specimens of amylose gave somewhat different curves from those drawn from the data in Table 5 although of the same type. Sometimes there was no appreciable fall in blue or red value till 80% hydrolysis. Sample K in Table 5 was a slightly 'denatured' amylose. The residue, when just over 90% hydrolysis was attained, possessed no blue or red colouring power, and was able to resist hydrolysis.

SUMMARY

1. Pure potato amylose is hydrolyzed by β -amylase at a steadily declining rate, without sudden velocity changes, until 100% of maltose has been formed.

2. If any 'denaturation' of the amylose has occurred, due to undue exposure to water, there is a break in the progress curve and the hydrolysis is incomplete.

3. Amylose crystallized from potato starch paste at pH 4, or from autoclaved paste at ordinary reaction, hydrolyzes faster than preparations crystallized from non-autoclaved pastes at pH 6 or 9.

4. The reaction obeys the formulation of Michaelis & Menten, but the value of K_m (expressed as percentage) varies widely with the preparations.

5. The shorter the molecular chains the more slowly they are hydrolyzed.

6. Evidence from iodine colorations suggests that amylose prepared by the crystallization methods contains no short-chain molecules (chain length < 12), and that none is formed until hydrolysis is far advanced.

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*

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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).