[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA.]

THE CONVERSION OF CINCHONINE AND QUININE INTO THEIR POISONOUS ISOMERS, CINCHOTOXINE AND QUINOTOXINE, AND THE RELATION OF THIS CONVERSION TO THE TOXICITY OF THE CINCHONA ALKALOIDS.[1]

BY H. C. BIDDLE.[2]

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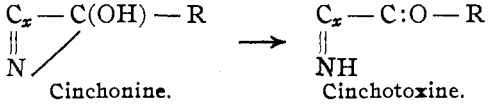
It was shown by v. Miller and Rohde,[3] in 1895, that prolonged heating of cinchonine with acetic acid at a temperature of 100° leads to the formation of a substance isomeric with cinchonine, to which, because of its poisonous properties, they gave the name of cinchotoxine. From its method of preparation and its low optical activity, they suspected this new substance to be identical with a similar isomeric body, cinchonicine, which had been obtained more than forty years before by Pasteur[4] through fusion of the salts of cinchonine, preferably the bisulfate, and which had subsequently been studied by a number of investigators.[5] Cinchotoxine, however, was obtained by v. Miller and Rohde in a crystallin form differing from that of cinchonicine. This discrepancy remained unexplained until five years later, when Brunner[6] showed that the substance is dimorphic in its crystalline habit and is in every way identical with the cinchonicine of Pasteur.

The chemical properties of cinchotoxine indicate that it is a secondary base containing a ketone grouping in the molecule.[7] Cinchonine itself is a tertiary base with a hydroxyl group in the molecule. The transformation of cinchonine into cinchotoxine may, consequently, be represented by the following molecular change:



Cinchonine. Cinchotoxine.

This rearrangement takes place in the so-called "second half" of the cinchonine molecule and is indicated somewhat more clearly in the following possible formulas of Rabe,[8] which, among others, have been suggested for cinchonine and cinchotoxine:

[1] Biddle, Preliminary report before the 42nd meeting of Am. Chem. Soc., San Francisco, July, 1910 (Abstract in *Science*, **32**, 486).

[2] The experimental work in this investigation has in part been carried out in conjunction with two of my students, O. L. Brauer and T. B. Kelly.

[3] Miller and Rohde, *Ber.*, **27**, 1187, 1279 (1894); **28**, 1056 (1895).

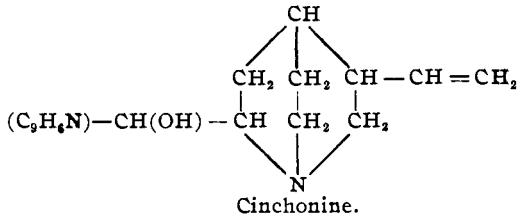
[4] Pasteur, *Jahrb.*, **1853**, 473; *Compt. rend.*, **37**, 110 (1853).

[5] Hesse, *Ann.*, **147**, 242 (1868); **166**, 277 (1873); **178**, 253 (1875). Howard, *J. Chem. Soc.*, **25**, 102 (1872). Roques, *Bull. soc. chim.*, [3] **13**, 1007 (1895).

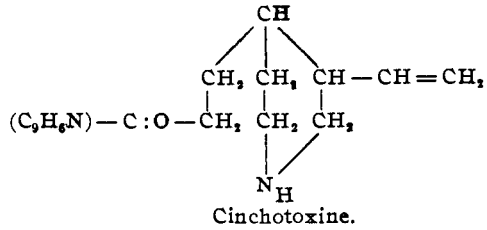
[6] v. Miller and Rohde, *Ber.*, **33**, 3214, 3220 (1900).

[7] *Ibid.*, **28**, 1056 (1895).

[8] Rabe, *Ann.*, **364**, 334 (1909); **373**, 88, 92 (1910).

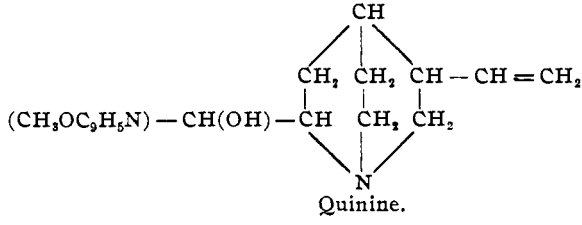


Cinchonine.

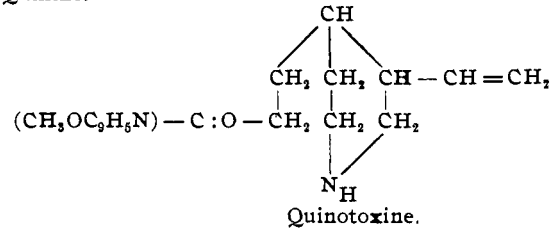


Cinchotoxine.

This peculiar molecular rearrangement to which cinchonine is subject at higher temperatures is not confined to this alkaloid. Other cinchona alkaloids[1] under similar conditions experience a like change. Thus, quinine, after prolonged boiling with acetic acid, is converted into a poisonous isomer, quinotoxine. The change in this case may be represented by the following formulas:



Quinine.



Quinotoxine.

Quite remarkable is the change in physiological properties experienced by the cinchona alkaloids after they have undergone this molecular rearrangement. Both cinchonine and quinine lose entirely their characteristic febrifugal action and become intensely poisonous. On this account, as has already been indicated, v. Miller and Rohde gave to the new products the names cinchotoxine and quinotoxine and likened their poisonous action to that of digitoxin, the highly poisonous constituent of digitalis leaves. According to the recent investigations of Hilde-

[1] v. Miller and Rohde, *Ber.*, 28, 1056 (1895).

brandt,[1] the specific action of cinchotoxine in warm-blooded animals is to induce violent convulsions, resulting in death if the dose is sufficiently large. The action of quinotoxine is similar, although less in intensity.

According to this investigator, the toxicity of cinchotoxine and quinotoxine is largely to be ascribed to the presence of the imide group in the molecule.

In the administration of the salts of cinchonine and of quinine as a febrifuge, physicians[2] not infrequently report cases in which the action of the drugs appears to be abnormal. Such abnormal action, if accounted for at all, has been generally ascribed to some idiosyncrasy of the patient. The thought occurred to me some time ago that the toxic action of cinchonine and quinine might be due to the formation in the system of small quantities of cinchotoxine and quinotoxine. With this thought in mind the following investigation was undertaken to determin with greater exactness the conditions under which cinchonine and quinine are converted into their poisonous isomers. The results obtained are significant. It has been found that acids of not too large dissociation constants, such as the organic acids, under suitable conditions, play a remarkable role as catalyzers in effecting the conversion of the cinchona alkaloids into their poisonous isomers. It has been found, furthermore, that these alkaloids give rise to their toxic isomers under conditions which may obtain in portions of the digestive tract, that under favorable conditions, indeed, partial conversion will take place at ordinary atmospheric temperatures.

Cinchotoxine.

1. Preparation and Properties of Cinchotoxine.—Cinchotoxine was prepared according to the method of v. Miller and Rohde[3] by heating at the boiling temperature for thirty-five hours a mixture of ten grams of cinchonine, ten grams of glacial acetic acid, and one hundred and thirty cc. of water. The brownish red solution when cooled was treated with excess of sodium hydroxide and the light brown oil which separated was extracted with ether. Instead of drying the ethereal solution with solid potassium hydroxide, as did v. Miller and Rohde, it was found more advantageous to effect dehydration by treatment with calcium carbide. Unchanged cinchonine being practically insoluble in dry ether is precipitated (except traces) when the solution is dried. The excess of ether was then evaporated off in a stream of dry hydrogen. The cinchotoxine is left as a light brown oil, which when well cooled gradually solidifies to a crystallin mass. After recrystallization from dry ether, the sub-

[1] Hildebrandt, *Archiv. fur. Ex. Path. u. Pharm.*, 59, 127 (1908).

[2] We are indebted to Dr. F. R. Woolsey, of Berkeley, for report on a number of these cases.

[3] v. Miller and Rohde, *Ber.*, 28, 1064 (1895).

stance melts with preliminary softening, as given by v. Miller and Rohde, at 58–59°.

The crystals give the characteristic reddish-purple coloration with an alkaline solution of diazobenzene sulfonic acid[1] and with nitrobenzene containing in solution dinitrothiophene.[2]

A few of the salts of cinchotoxine are described in the literature.[3] The hydriodide, $C_{19}H_{22}N_2O.HI$, was prepared by neutralizing cinchotoxine with hydriodic acid. The salt as recrystallized from alcohol is obtained in large brown prisms. A nitrogen determination led to the following results:

0.3 gram substance; 17.7 cc. N_2 at 24° and 755.5 mm.
Calculated for $C_{19}H_{22}N_2O.HI$: N, 6.65; found, 6.6%.

The following salts prepared by neutralizing cinchotoxine with the corresponding acid and purified by reerystallization from alcohol were not analyzed:

Cinchotoxine sulfate, white, crystalline plates, m. p. 56°.

Cinchotoxine lactate, colorless plates, m. p. 56°.

Cinchotoxine propionate, white plates, m. p. 59°.

2. Conversion of Cinchonine into Cinchotoxine at 100°.—In the conversion of cinchonine into cinchotoxine by fusion of the bisulfate, Pasteur employed temperatures of 160° and above. Similarly, Hesse effected the transformation at temperatures above 130°. As we have seen, a practically quantitative conversion of the alkaloid into its poisonous isomer is brought about by heating cinchonine with acetic acid at 100°. The suggestion naturally presents itself that possibly the particular acid concerned may have some influence upon this molecular change. To determin this point a series of experiments was carried out in which like quantities of cinchonine were heated at 100° for the same length of time with excess of different acids and the actual amount of conversion produced was determined by isolating and weighing the cinchotoxine formed. It was found that some change was effected in the case of all the acids used, but that the amount of conversion varied between the widest limits. Organic acids such as acetic and propionic gave almost quantitative change, while in the case of the highly ionized mineral acids, such as hydrochloric acid, the cinchotoxine produced was simply a trace.

These results seemed to indicate the possibility, on the one hand, of a strong catalyzing influence on the part of certain organic acids, and on

[1] Penzoldt and Fisher, *Ber.*, **16**, 657 (1883). v. Miller and Rohde, *Ibid.*, **28**, 1058 (1895).

[2] v. Meyer, *Ibid.*, **17**, 2778 (1884); **18**, 533 (1885). v. Miller and Rohde, *Ibid.*, **33**, 3223 (1900).

[3] Hesse, *Ann.*, **178**, 256 (1875). Roques, *Bull. soc. chim.*, [3] **13**, 1007 (1895). Howard, *J. Chem. Soc.*, **25**, 102 (1872).

the other hand an inhibiting influence due to the concentration of the hydrogen ions. In order that further light might be thrown upon these points, a series of experiments was carried through with different acids in solutions of known concentration with respect to the hydrogen ion. To determin the concentration of the hydrogen ions, recourse was had to the colorimetric scheme proposed by E. Salm,[1] his series of standard solutions varying from 2 N hydrogen ion to 1×10^{-10} N hydrogen ion being used as a basis of comparison. The following eight indicators were employed in determining the normality of the hydrogen ion: cochineal, hematein, congo red, litmus, methyl orange, methyl violet, *p*-nitrophenol, phenacetolin, phenolphthalein, rosolic acid and sodium alizarin sulfonate.

In the experiments whose results are tabulated below, the mode of procedure was uniformly as follows: The acid was added to 5 g. of cinchonine in 65 cc. of water. The mixture was then heated for 48 hours on an electric heater at 98–102°. The cooled solution was thereupon treated with excess of sodium hydroxide and the cinchotoxine and unchanged cinchonine extracted with ether, care being taken to follow like conditions of procedure in each case. After the ethereal solution had been freed from moisture and all but traces of dissolved cinchonine by treatment with calcium carbide, the ether was evaporated off and the weight of the dry residue determined, when it had become constant. This residue presented, in every case in which there was sufficient for examination, the characteristic properties of cinchotoxine.

During the process of heating, the solutions in all cases were more or less colored from the formation of resinous matter insoluble in ether. In general those solutions were least colored in which the lesser amount of cinchotoxine was formed. In those cases in which practically a quantitative conversion to cinchotoxine was effected slight quantities of an oily resin insoluble in ether were frequently obtained. These, in many cases, contained traces of unchanged cinchonine.

It should be stated that the results following are to be regarded only as roughly quantitative, the object being to determin the influence of the reagent in question on the general course of the reaction. Furthermore, the colorimetric scheme of Salm is open to criticism as to its accuracy when used in the presence of such substances as the salts of cinchonine. The observed concentrations of the hydrogen ions are, consequently, to be regarded as presenting only approximate values.

The results obtained, however, clearly reveal a most remarkable case of catalysis concerning which I presented a preliminary report before the section in organic chemistry of the 42nd general meeting of the American Chemical Society, held in San Francisco, July, 1910.[2]

[1] *Z. physik. Chem.*, **57**, 471 (1906).

[2] In November, 1910, Rabe reports (*Ber.*, **43**, 3308) certain work carried out

TABLE I.

	Acid.	Ratio of cinchonine (1 mol.) to equivalent of acid.	Approximate concentration of hydrogen ion.	Dissociation constant K.	Cinchotoxine formed. Grams.	Unchanged cinchonine recovered. Grams.	Amount of conversion.
1	Hydrochloric.....	1 : 4	1×10^{-1}	....	None	4.7	None
2	Oxalic,.....	1 : 4.5	1×10^{-2}	....	0.1	4.7	2.0%
3	Dichloracetic.....	1 : 3.5	1×10^{-2}	5.1	0.1	4.8	2.0%
4	Monochloracetic...	1 : 3	1×10^{-2} / 1×10^{-3}	0.155	0.5	4.1	10.0%
5	Tartaric.....	1 : 4	1×10^{-3}	0.097	4.6	None	Quantitative
6	Malic.....	1 : 4.5	1×10^{-3}	0.0395	4.7	"	"
7	Formic.....	1 : 8	1×10^{-3}	0.0214	4.6	"	"
8	Lactic.....	1 : 4	1×10^{-3}	0.0138	4.7	"	"
9	Citric.....	1 : 4	1×10^{-3} / 1×10^{-4}	....	4.7	"	"
10	Acetic.....	1 : 20	1×10^{-4}	0.0018	4.6	"	"
11	Acetic.....	1 : 5	1×10^{-4}	0.0018	4.7	"	"
12	Hydrochloric.....	1 : 1.2	1×10^{-4}	....	0.12	4.7	2.4%
13	Hydrochloric (Cinchonine hydrochloride).....	1 : 1	1×10^{-6}	....	0.15	4.75	3.0%
14	Sulfuric (Cinchonine sulfate).....	1 : 1	1×10^{-6}	....	0.2	4.6	4.0%
15	Tartaric (Cinchonine tartrate, 5 g. of cinchonine in 175 cc. of solution)	1 : 1	1×10^{-6}	....	0.5	4.2	10.0%
16	Tartaric (Cinchonine tartrate, 5 g. of cinchonine in 175 cc. of solution) and sodium potassium tartrate (5 mol.)....	1 : 1 : 10	....	....	0.5	4.3	10.0%

From these experiments it is seen that cinchonine gives rise to cinchotoxine when salts of the alkaloid either with or without excess of acid are heated in aqueous solution at 98–102°. With excess of acid the speed of conversion, or the amount of change in unit time, in general increases with acids of decreasing dissociation constant. With such acids as hydrochloric, oxalic, dichloracetic and monochloracetic, the rate of conversion is comparatively slow, nor is this rate of conversion much affected by lowering the concentration of the hydrogen ion of a strong acid such as hydrochloric, as is shown in Expt. 12. A similar slow rate of con-

by McMillan on the accelerating action of weak a ids, such as many of the organic acids, on the conversion of cinchonine into cinchotoxine. He has, apparently, overlooked the fact that four months before I brought out the same points, in addition to others, in my preliminary report before the American Chemical Society in San Franci co and that an abstract of said report was published in the October number of *Science*, 32, 486 (1910).

version obtains in the case of the neutral salts, such as the hydrochloride and sulfate. With excess of acids of small dissociation constant such as tartaric, malic, formic, lactic, citric, and acetic, the rate of change is enormously increased so that within 48 hours, under the conditions of the experiment, the conversion is quantitative. This increase in speed of conversion, apparently, does not take place in case of the monacid salts of cinchonine with these acids even in the presence of excess of the neutral salts of such acids, as is shown in Expts. 15 and 16.

In order that more definit information might be obtained regarding the comparative speed of conversion, experiments were carried out whose results are recorded in Table II. 5 g. of cinchonine were used in each case with 5 equivalents of acid in 65 cc. of water.

TABLE II.

			Time of heating.					
			6 hours.		24 hours.		48 hours.	
	Acid.	K.	Cinchotoxine formed. Grams.	Cinchonine recovered. Grams.	Cinchotoxine formed. Grams.	Cinchonine recovered. Grams.	Cinchotoxine formed. Grams.	Cinchonine recovered. Grams.
1	Hydrochloric.....	...	...	...	...	...	None	4.6
2	Monochloracetic..	3.155	...	...	0.2	4.5	0.3	4.4
3	Tartaric.....	0.097	1.7	3.0	4.5	0.2	4.7	None
4	Acetic.....	0.0018	2.4	2.2	4.7	None	...	...

The enormous increase in the speed of conversion observed in passing from monochloracetic to tartaric acid seems remarkable in view of the little difference between the dissociation constants of these acids. But these results are in accord with those found in Table I, where, as is seen, a marked change occurs at this point. In the case of the acetic acid, as may be noted, conversion to the extent of 50% occurs within 6 hours.

TABLE III.

	Acid.	Ratio of cinchonine (1 mol.) to equivalent of acid.	Approximate concentration of hydrogen ion in the solution.	Cinchotoxine formed. Grams.	Unchanged cinchonine recovered. Grams.	Amount of conversion.
1	Hydrochloric.....	1 : 4	1×10^{-1}	None	4.7	None
2	Hydrochloric and acetic..	1 : 4 : 5	1×10^{-1}	0.2	4.8	4.0%
3	Dichloracetic.....	1 : 3.5	1×10^{-2}	0.1	4.8	2.0%
4	Dichloracetic and acetic..	1 : 3.5 : 5	1×10^{-2}	0.18		3.6%
5	Hydrochloric.....	1 : 1.2	1×10^{-4}	0.12	4.7	2.4%
6	Hydrochloric and acetic..	1 : 1.2 : 5	1×10^{-4}	4.2	...	84.0%
7	Hydrochloric (Cinchonine hydrochloride).....	1 : 1	1×10^{-6}	0.15	4.6	4.0%
8	Hydrochloric (Cinchonine hydrochloride) and sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$)..	1 : 1 : 4	1×10^{-6}	4.7	None	Quantitative

The catalyzing influence of the weaker acid appears also in the case of the *monacid* salts of cinchonine with the more strongly dissociated

acids, such as cinchonine hydrochloride. This is shown in the preceding results which were obtained under the same conditions of heating as those given in Table I.

As may be seen from these results when the strongly dissociated acid, such as hydrochloric, is present in sufficient quantity to form the di-acid salt of cinchonine or is in excess of this amount, the less dissociated acid apparently exerts a weak catalytic action but the acceleration is practically negligible. If, however, the strongly dissociated acid is present only in sufficient quantity to form the monacid salt of cinchonine or is but slightly in excess of this quantity, presence of sufficient quantity of the less dissociated acid suffices to effect practically quantitative conversion of cinchonine into cinchotoxine within 48 hours. The rate of conversion is, consequently, apparently closely dependent upon the condition of the less basic or second nitrogen atom in the cinchonine molecule.

From these considerations it might be suggested that an explanation of this remarkable case of catalysis is to be sought under the phenomena of hydrolysis. As a study of the conditions of this conversion is being continued with the idea of more fully determining its nature, I wish for the present to withhold an interpretation of the reaction until further data is obtained.

3. Conversion of Chichonine into Cinchotoxine at 36°.—The formation of cinchotoxine from cinchonine may be effected in the presence of the preceding catalyzing agents at temperatures as low as 36°, or at temperatures corresponding to those occurring in the body. The conversion at these temperatures is in every case slight—a matter which renders somewhat tedious the separation of the cinchotoxine from the unchanged cinchonine in attempting to determin the amount of the former produced. Fortunately a ready method for the detection of cinchotoxine is found in the two color tests already referred to, *viz.*, that with an alkaline solution of diazobenzene sulfonic acid and that with a solution of dinitrothiophene in nitrobenzene.

The Diazobenzene Sulfonic Acid Reagent.—This is most conveniently prepared by making a freshly saturated aqueous solution of the free acid. The solution to be tested is added to 2–3 cc. of the solution of the reagent, after the latter has been rendered alkaline by the addition of a little caustic soda. If cinchotoxine is present, the solution is colored within 5–15 minutes, the color varying from a light pink to a deep reddish violet, according to the amount of cinchotoxine present. The reaction is determined apparently by the presence in cinchotoxine of the carbonyl group, as it is yielded by aldehydes and, in general, somewhat less pronouncedly by ketones.[1]

[1] Penzoldt and Fischer, *Ber.*, 16, 657 (1883). v. Miller and Rohde, *Ibid.*, 28, 1058 (1895).

This color test, unlike the other, may be used directly in testing the alkaline solution from which the cinchonine and cinchotoxine have been precipitated by the addition of an alkali. If much cinchotoxine has been formed, sufficient will remain in solution to give a distinct reaction. Small amounts, however, may escape detection in this application of the reagent.

The Dinitrothiophene Reagent.—This was prepared by dissolving dinitrothiophene in pure nitrobenzene (1 : 200). Ordinary impure nitrobenzene frequently contains sufficient dinitrothiophene to serve as an indicator. To 1–2 cc. of the reagent is added a few drops of alcohol and then the substance to be tested. Presence of cinchotoxine is indicated by a color change varying from light reddish yellow to a deep wine-red (with at first a distinct violet tint) according to the amount of cinchotoxine present. Brunner[1] considered the reaction to depend upon the imide group in cinchotoxine, but it seems more reasonable to consider it as due to the pronounced basic character of this substance, since the reaction is given vigorously by such strong bases as sodium hydroxide and triethylamine, and much less vigorously by weaker bases such as aniline and ethyl aniline. In employing the dinitrothiophene reagent, the cinchotoxine was freed from sodium hydroxide and all but traces of co-precipitated cinchonine by extracting with ether. The ethereal solution, after dehydration with calcium carbide, was evaporated to dryness and the residue taken up with a little ether, and was added directly to 2–4 drops of the reagent together with a little alcohol.

To determin the influence of the various acids upon cinchonine at lower temperatures, a series of experiments was carried out at 36° similar to those conducted at 100°. In these experiments the method of procedure was in general like that followed in the preceding case. The bases were precipitated with excess of sodium hydroxide and both the alkaline solution and the unchanged cinchonine carefully extracted with ether. The cinchotoxine obtained on evaporation of the dried solution was freed from traces of cinchonine by solution in a little dry ether. It was thus possible to detect and to isolate even traces of cinchotoxine from a large quantity of unchanged cinchonine. The cinchotoxine obtained at the lower temperature gives the vigorous color reactions with dinitrothiophene in nitrobenzene and with diazobenzene sulfonic acid and in all its properties is identical with that obtained at the higher temperature.

In making the colorimetric tests, the cinchotoxine was ordinarily dissolved in a little pure, dry ether and this solution was added to the reagent in question. The ether employed throughout the work was carefully freed from vinyl alcohol[2] and other impurities which are found in

[1] v. Miller and Rohde, *Ber.*, **33**, 3223 (1900).

[2] Poleck, Thümmel, *Ibid.*, **22**, 2863 (1889).

ordinary ether and which would give color reactions with an alkalin solution of diazobenzene sulfonic acid.

In the experiments (excepting the last) recorded in the following table, the acid in each case was dissolved in 10 cc. of water and the aqueous solution added to 2½ g. of cinchonine. The temperature was then maintained at 36° for 48 hours:

TABLE IV.

	Acid.	Ratio of cinchonine (1 mol.) to equivalent of acid.	Cinchotoxine formed.	Approximate per cent of conversion.	Remarks.
1	Hydrochloric	1 : 5	None		Solution scarcely colored by heating
2	Hydrochloric	1 : 5	"		Solution scarcely colored by heating
3	Sulphuric (Cinchonine bisulfate)	1 : 2	"		
4	Formic	1 : 6	Slight amount		Solution strongly colored by heating
5	Acetic	1 : 5	" (0.02 g.)	0.8	
6	Acetic	1 : 5	" (0.025 g.)	1.0	
7	Propionic	1 : 5	" (0.04 g.)	1.6	
8	Malic	1 : 7	"		
9	Lactic	1 : 4	" (about 0.006 g.)	0.2	
10	Citric	1 : 8	Very slight amount		Solution somewhat colored by heating
11	Sulfuric (Cinchonine sulfate; 4 g. in 30 cc. of water) and acetic.	1 : 1 : 5	Slight amount (about 0.005 g.)	0.2	

As is seen, the results accord in general with those obtained with the same acids at 100°, the differences being those of degree rather than of character. The conversion, however, is slight, in no case reaching 2%.

The following results were obtained by the action of acetic acid and hydrochloric acid and mixtures of these two acids, in each case on 2 g. of cinchonine in 10 cc. of water, the temperature being maintained at 36° for 48 hours:

TABLE V.

	Acid.	Ratio of cinchonine (1 mol.) to equivalent of acid.	Cinchotoxine formed.
1	Acetic	1 : 10	Small amount (about 0.03 g.)
2	Acetic	1 : 5	" (about 0.01 g.)
3	Acetic and hydrochloric	1 : 5 : 1.6	" (less than 0.006 g.)
4	Acetic and hydrochloric	1 : 2.5 : 5	None
5	Hydrochloric	1 : 5	None
6	Hydrochloric	1 : 10	None

These results likewise are in perfect accord with those obtained at 100°. Hydrochloric acid, which at the higher temperature shows no change, at the lower temperature gives none detectable. Acetic acid, which at the higher temperature gave a large conversion, shows here the same general catalyzing action. Here, as at the higher temperature, with a mixture of the two acids, an excess of hydrochloric acid inhibits the catalyzing action of the acetic acid, while in the presence of only a little hydrochloric acid (less than 2 acid equivalents to the cinchonine molecule), a partial catalysis takes place.

4. Effect of Dilution on the Conversion of Cinchonine to Cinchotoxine.—The catalyzing influence of the organic acid is not destroyed even on large dilution as is shown in the following results, which were obtained by maintaining the temperature at 36° for 48 hours:

TABLE VI.

	Acid.	Amount of cinchonine. Grams.	Water. cc.	Percentage of cinchonine in the solution.	Cinchotoxine formed. Gram.	Per cent conversion.
1	Acetic (7.4 cc., 99.9%).....	10.0	20	26.3	0.1	1.0
2	Acetic (2 cc., 99.9%).....	2.5	10	17.1	0.02	0.8
3	Acetic (2 cc., 99.9%).....	2.0	200	0.98	0.0075	0.37

5. Effect of Duration of Time on the Conversion of Cinchonine into Cinchotoxine.—The amount of cinchotoxine formed from a given quantity of cinchonine at 36° by the action of organic acids apparently increases but slowly with continuance of the action. Appreciable conversion is usually easily shown within 24 hours or less. Prolonged maintenance of temperature leads to the formation of considerable resin and it is probable, as will be shown later, that the cinchotoxine is in part polymerized into this.

The following experiments were carried out at 36°, the acid used being acetic:

TABLE VII.

Amount of cinchonine. Grams.	Ratio of cinchonine (1 mol.) to equivalent of acid.	Water. cc.	Time.	Cinchotoxine formed.	Approximate conversion. Per cent.
2	1 : 5	10	48 hrs.	0.02	1.0
2	1 : 5	10	15 days	0.085	4.2
20	1 : 5	100	3.5 mo.	about 1 g.	5.0

6. Action of Organic Substances other than Acids upon Cinchonine.—As shown by Howard,[1] cinchonine, when heated with glycerine to 180° and above this temperature, is converted into cinchotoxine. The influence of neutral substances at lower temperatures is consequently of interest.

Four grams of cinchonine were heated in a solution of absolute alcohol at the temperature of the boiling alcohol for 48 hours. The solution, which remained perfectly clear and colorless, was cooled and separated

[1] Howard, *J. Chem. Soc.*, 25 102 (1872).

from the excess of cinchonine, which crystallized out. The residue remaining on evaporation of the alcohol was found to be free from cinchotoxine.

The effect of alcohol upon a salt of cinchonine was determined by heating 5 g. of cinchonine hydrochloride dissolved in a mixture of equal parts of water and absolute alcohol, the solution being maintained at the temperature of boiling for 48 hours. A small amount (about 0.04 g.) of cinchotoxine was formed, but the quantity was less than that obtained from heating cinchonine hydrochloride in aqueous solution alone at about the same temperature for the same length of time.

Experiments carried out with cinchonine in acetone, chloroform, and mixtures of chloroform and alcohol gave no evidence of the formation of cinchotoxine. These solvents, consequently, do not act as catalyzers under such conditions to effect the conversion of cinchonine into cinchotoxine.

7. *The Action of Sunlight on the Salts of Cinchonine.*—The salts of cinchonine and of the cinchona alkaloids in general are profoundly affected by the sunlight—a matter which was noted by Pasteur[1] as early as 1853 and to which later Hesse[2] called attention. These investigators suspected that the changes produced were similar to those effected at elevated temperatures, but they failed to confirm their suspicions.

A study of the behavior of the salts of cinchonine under the influence of sunlight discloses the fact that the changes produced are of the same nature as those already considered. Three solutions of 100 cc. each containing in each case 1 g. of cinchonine dissolved in four molecules of hydrochloric, acetic and citric acids, respectively, were exposed to the direct action of the sunlight. Within eight hours the solutions containing the cinchonine acetate and citrate were deep brown in color from the formation of resinous matter, while the solution containing the hydrochloride was unchanged. After twenty hours' exposure, the brown of the first two had deepened in intensity, while the last solution had only begun to show a trace of color. Precipitation of the cinchonine, extraction with ether, etc., as before, revealed the presence of traces of cinchotoxine in the case of the acetic acid and the citric acid, but none in the case of the hydrochloric acid.

Solutions of the salts of cinchonine are affected in the same way by diffused light, although in this case the action is naturally much slower. Solutions of the alkaloid with the above acids were allowed to stand in diffused light at ordinary temperatures for about 60 days. The color changes corresponded with those obtained by the more rapid action of the direct sunlight.

[1] Pasteur, *Compt. rend.*, **37**, 110 (1853).

[2] Hesse, *Ann.*, **166**, 275 (1873).

The action of sunlight upon the salts of cinchonine results in the formation of much resinous matter. It seems probable that this is derived not directly from the alkaloid but from cinchotoxine formed as an intermediate product. Salts of the latter base are even more sensitive to sunlight than those of cinchonine. Like those of cinchonine, the salts of cinchotoxine with organic acids in the presence of excess of acid are converted into resinous matter in the sunlight much more rapidly than those with such acids as hydrochloric. Thus, a solution of cinchotoxine in acetic acid exposed to the sunlight becomes deep brown in color before a corresponding solution of the alkaloid in hydrochloric acid is much affected.

8. Conversion of Cinchonine into Cinchotoxine, Apparently not Reversible.—The fact that prolonged action of the organic acids at 36° but slowly increases the amount of cinchotoxine formed naturally leads to the inference that the action is reversible, a condition of equilibrium having been approached. The quantitative conversion at 100°, however, does not favor this view. Furthermore, previous attempts[1] as well as attempts made by myself to reverse the action have thus far been unavailing. It is possible that we are dealing here with a case of false equilibrium[2]—a matter, however, which cannot be determined without a more extensive study of the conditions underlying this molecular change.

A study of the possible reversal of cinchotoxine to cinchonine is much complicated by the large number of stereoisomeric substances which may arise from cinchonine because of the existence in the molecule of four dissimilar asymmetric carbon atoms (see formula of Rabe). Indeed, nearly twenty such isomers have been described as obtained from cinchonine by the action of acids or alkalies under varying conditions of temperature.[3] It may be that future study will decrease (as more recent investigation appears to be doing) the number of these isomers by showing that some of them are identical with one another. The number of isomers actually possible is naturally increased, if one includes with these cinchotoxine and the possible stereoisomers of this substance.

While the direct reversal of cinchotoxine to cinchonine appears improbable, it is interesting to note that Rabe[4] has recently, by an indirect method, succeeded in converting the former substances into the latter, thus solving one step in the partial synthesis of cinchonine.

[1] Koenigs, *Ber.*, 40, 2875 (1907).

[2] Duhem-Burgess, "Thermodynamics and Chemistry," 1903, p. 369.

[3] Hesse, *Ann.*, 205, 330 (1880); 227, 153 (1885); 243, 131 (1888); 260, 213 (1890); 266, 245 (1891); 267, 142 (1892); 276, 88 (1893). Skraup, *Ann.*, 201, 291 (1880); *Ber.*, 25, 2909 (1892); *Monatsh.*, 12, 431 (1891); 18, 411 (1897); 20, 571, 585 (1899); 21, 512 (1900); 22, 171, 253, 1083, 1097 (1901). Skraup and Zwerger, *Monatsh.*, 21, 535 (1900); 23, 455 (1902). Zwerger, *Monatsh.*, 24, 119 (1903). Comstock and Königs, *Ber.*, 20, 2510 (1887), and many others.

[4] Rabe, *Ber.*, 44, 2088 (1911).

Quinotoxine.

9. Preparation and Properties of Quinotoxine.—Quinotoxine, or quinicine, bears to quinine (the methoxyl derivative of cinchonine) the same relation that cinchotoxine bears to cinchonine. It was first obtained by Pasteur[1] in 1853 by heating quinine with dilute sulfuric acid to 120–130°. In general, the conversion of quinine into its poisonous isomer has been effected under conditions quite parallel with those under which cinchonine yields cinchotoxine.[2]

Quinotoxine was prepared from quinine by the same general process used in obtaining cinchotoxine from cinchonine, *viz.*, the heating of the alkaloid with acetic acid for 35 hours at 100°. As obtained from its ethereal solution, it forms a yellowish brown oil, which does not crystallize. On standing in the air it is, after some time, converted into an amorphous resinous mass, having a rough melting point near 60°. It is slightly soluble in water, readily so in alcohol, ether, and chloroform. Like cinchotoxine, quinotoxine contains ketone oxygen and an imide group; it responds like that compound to the color tests with diazobenzene sulfonic acid and dinitrothiophene in nitrobenzene; and in its reactions in general, it shows itself to be the analog of cinchotoxine.

10. The Conversion of Quinine into Quinotoxine at 100°.—The catalyzing action of various acids upon quinine at 100° closely resembles the corresponding action of these acids upon cinchonine. The experiments whose results are given in the following table were carried out as in the former case (see page 505). Since quinine, unlike cinchonine, is somewhat soluble in dry ether (approximately 1 part in 18 parts at 18°), the separation of the quinotoxine from unchanged quinine presents additional difficulties. The results obtained are consequently open to a greater degree of experimental error, particularly in cases where much quinine remained unchanged. A moment's consideration of these results, however, shows their close accordance with the like results obtained in the case of cinchonine:

TABLE VIII.

Amount of quinine used, 5 g.; temperature, 98–102°; time of heating, 48 hours.

Acid.	Ratio of quinine (1 mol.) to equivalent of acid.	Dissociation constant. K.	Quinotoxine formed. Grams.
Hydrochloric	1 : 4	....	None
Formic	1 : 8	0.0214	4.5
Lactic	1 : 4	0.0138	4.6
Acetic	1 : 5	0.0018	4.5

11. The Conversion of Quinine into Quinotoxine at 36°.—In the presence

[1] Pasteur, *Compt. rend.*, **37**, 110, 166 (1853); *Jahrb.*, 1853, 473.

[2] Hesse, *Ann.*, **166**, 277 (1873); **243**, 148 (1888). v. Miller and Rohde, *Ber.*, **33**, 3228 (1900).

of the ordinary organic acids, such as formic, propionic, butyric and lactic, quinine like cinchonine yields within 48 hours, at 36°, an appreciable quantity of its poisonous isomer, quinotoxine. In the presence of excess of sulfuric or hydrochloric acid under the same conditions there is no detectable conversion.

In a few cases the quinotoxine formed at 36° was freed from all but traces of unchanged quinine, but the difficulty of separating small quantities of the substance from large amounts of unchanged base rendered the results of little quantitative value. The quinotoxine obtained, however, possesses all the properties of that formed at 100° and the degree of conversion is of like order to that occurring in the case of cinchonine under the same conditions.

12. The Employment of the Cinchona Alkaloids in Medicin.—From the results of these investigations it is very clear that cinchotoxine and quinotoxine may be formed in small quantities in the system during the administration of the salts of cinchonine or of quinine. As shown by Hildebrandt,[1] these isomers have no febrifugal properties while their poisonous character is most pronounced. Although the amount of conversion would apparently be slight, yet the question is naturally raised as to whether the administration of the cinchona alkaloids under these circumstances becomes in any way a menace to the health of the patient. While Hildebrandt has studied the action of cinchotoxine and quinotoxine upon the lower warm-blooded animals, such as rabbits and cats, unfortunately, we have no data regarding their action upon the human system, unless the so-called abnormal behavior of the salts of cinchonine and quinine be regarded as furnishing such.

In medicin, cinchonine and quinine are sometimes administered in the form of the free alkaloid; more frequently, however, in the form of their salts. Doses of varying quantity are given: from 1–2 grains (0.06–0.12 g.) as a tonic, to 15–30 grains (1–2 g.) as an antiperiodic. The possibility of a 1–2% conversion of the alkaloids into their poisonous isomers in the presence of some such catalyzing agent as acetic acid, malic acid, citric acid, or lactic acid follows naturally from the experimental evidence adduced. Foods such as sour milk, acid fruits and the like, would, consequently, favor such conversion.

It seems most probable, from these considerations, that the abnormal behavior of the salts of cinchonine or of quinine, when these alkaloids are administered as a medicin, finds its explanation in their partial conversion under favorable conditions, into their poisonous isomers.

13. Summary of Results.—1. Cinchonine and quinine, when heated at 98–102° in the form of their salts in aqueous solution, either with or

[1] Hildebrandt, *Archiv. Exper. Path. Pharm.*, **59**, 127 (1908).

without excess of acid, give rise to their poisonous isomers, cinchotoxine and quinotoxine.

2. In a general way, the rate of conversion of these alkaloids in the presence of acids increases with acids of decreasing dissociation constant. By changing the nature of the acid used and consequently the concentration of the hydrogen ion, the rate of conversion can be caused to vary between the widest limits. Thus, in the presence of excess of such acids as hydrochloric the rate of conversion may be so reduced as to give no detectable change after heating at 98–102° for 48 hours. On the other hand, in the presence of acids of small dissociation constant, such as acetic and propionic, the rate of conversion may be so accelerated under the same conditions as to lead to quantitative conversion in 24 hours.

3. The rate of the conversion of these alkaloids when heated at 98–102° in their monacid salts with various acids and in their diacid salts with strong acids is slow. The rate of this conversion in the case of the monacid salts is enormously increased by the introduction of an acid of small dissociation constant such as acetic acid. In the case of their diacid salts with the strong acids, however, such introduction of the weak acid is of but slight effect.

4. Neutral substances, such as alcohol and chloroform, do not, under ordinary conditions, act as catalyzers in accelerating the conversion of these alkaloids into their isomers.

5. The salts of cinchonine and quinine undergo similar conversion when kept in solution at a temperature of 36°, the only noteworthy difference from that effected at 98–102° being the diminished rate of conversion. Thus, with such acids as hydrochloric and sulfuric, the conversion in 48 hours is too slight to be detected, while in the presence of the little dissociated organic acids, such as acetic and propionic, a conversion closely approaching 2% has been observed within the same length of time.

6. In the sunlight at ordinary temperatures the salts of cinchonine and quinine in solution undergo changes similar to those effected by rise of temperature. The cinchotoxine and quinotoxine, in this case as at more elevated temperatures, give rise to resinous matter, which colors the solutions.

7. The conditions under which cinchotoxine and quinotoxine are produced indicate the possibility of the formation of these substances in the body in the administration of cinchonine and quinine as remedial agents and consequently afford a reasonable explanation of cases of so-called "quinine poisoning."

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