[CONTRIBUTION FROM THE DEPARTMENT OF RESEARCH IN PURE CHEMISTRY, MELLON INSTITUTE OF INDUSTRIAL RESEARCH]

Cinchona Alkaloids in Pneumonia. I. Miscellaneous Alkaloids and Some Hydrocupreine Ethers

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There has been little advancement in the chemotherapy of pneumonia since the early work of Morgenroth and others on hydrocupreine ethers.[1] However, since serum therapy has been only partially successful, a chemotherapeutic approach to the pneumonia problem would appear to be desirable. Many physicians who have dealt with the disease believe that the attack should be directly on the pneumococcus. Accordingly, for the past three years, studies have been carried on in this Laboratory which have had as their purpose the discovery of drugs suitable for the treatment of this disease. The research is being conducted in collaboration with Drs. W. W. G. Maclachlan, H. H. Permar, John M. Johnston and J. R. Kenny of the Mercy Hospital, Pittsburgh, Pa. This medical staff has reported some preliminary biological results before the Association of American Physicians, Atlantic City, May 1, 1934.[2] Further results will be reported by them as the work progresses.

The work to date has been wholly concerned with the preparation and study of cinchona derivatives. Working with a series of hydroquinine homologs,[1] Morgenroth showed that pneumococcicidal activity reached a maximum in ethylhydrocupreine (optochin). Higher alkyl derivatives showed a lower bactericidal action toward the pneumococcus, although some of these, such as isoamyl and isooctyl hydrocupreine were very effective against other organisms.[3] While optochin is highly effective against the pneumococcus, it has some toxic properties which detract from its value in clinical use. These will be mentioned below. Our purpose was to prepare substances of lower toxicity than optochin, which would maintain high pneumococcicidal activity.

It was planned, therefore, to make a preliminary survey of all the natural and synthetic cinchona derivatives which were available, in order to have leads in as many directions in this field as possible; then because of the high specific action of optochin, to investigate a variety of ethyl ethers of isomeric or modified alkaloids; and further to make such modifications in the ethyl group as seemed practical.

Preliminary Examination of Miscellaneous Cinchona Alkaloids.—Among the naturally occurring alkaloids: quinine, quinidine, cupreine, cinchonine, cinchonidine and their hydrogenated derivatives, no suitable compound was expected and none was found. Hydroquinine was the only substance in this group which sterilized pneumococcus cultures in concentration of 1:50,000. It was ineffective in higher dilutions.

The new stereoisomers of quinine: epiquinine and epiquinidine, described by Rabe,[4] were also prepared and tested. Neither of these substances was of value. Epiquinidine sterilized in dilutions of 1:50,000 but failed in higher dilutions. It is interesting to note that toxicity experiments carried out by Professor W. T. Dawson, Department of Pharmacology in the Medical School, University of Texas, showed epiquinine to be much the most toxic of the group—quinine, epiquinine, epiquinidine, quinidine—to guinea pigs.

An increase in pneumococcicidal activity over quinine has already been observed for isoquinine.[5] The present preliminary results using isoquinine indicate that it has greatly lowered toxicity compared to optochin, together with moderate efficiency in protecting mice from pneumococcic infection, in spite of its rather low disinfecting power *in vitro*.

The following group of compounds shows a very low pneumococcicidal power *in vitro:* hydroxyhydroquinine,[6] from the addition of water to the vinyl group of quinine; quitenine, a carboxylic acid formed by oxidation of the vinyl group of quinine, and its ethyl ester; quitenidine,

(1) (a) Morgenroth and Levy, *Berlin klin. Wochenschr.*, **48**, 1560, 1979 (1911); (b) Morgenroth, *ibid.*, **51**, 1829, 1865 (1914); (c) Morgenroth and Brunke, *Deutsch. med. Wochschr.*, **40**, 539 (1914); (d) Moore, *J. Exptl. Med.*, **22**, 269 (1915).

(2) Maclachlan, Permar, Johnston and Kenny, *Am. J. Med. Sci.*, **188**, 699 (1934).

(3) Morgenroth and Tugendreich, *Biochem. Z.*, **79**, 257 (1917).

(4) Rabe, *Ann.*, **492**, 242 (1932).

(5) Gundel and Seitz, *Z. Immunitätsforschung*, **80**, Heft 3/4, 240 (1933).

(6) Giemsa and Oesterlin, *Ber.*, **64**, 57 (1931). Unpublished experiments in this Laboratory have shown that two hydroxy-hydroquinines, as well as isoquinine and possibly niquine, are formed by the action of cold concentrated sulfuric acid on quinine.

from the oxidation of quinidine, a diastereoisomer of quitenine; niquine, an isomerization product of quinine in which the C—N bond of the bridge chain in the quinuclidine ring has been broken. No animal tests of these substances have been made as yet. Cupreine, ethylcupreine, hydrocupreine, hydrocupreidine and ethylhydrocupreidine have also been examined. Ethylcupreine is the only interesting substance in this group. It showed fair pneumococcicidal activity *in vitro* associated with moderate efficiency in protecting mice from pneumococcic infections. It was, however, more toxic than some of the more efficient drugs to be described in this and following papers.

Hydrocupreine Ethers.—While ethylhydrocupreine, which has been a standard reference compound for the present chemotherapeutic study, has interesting practical applications, its use in doses large enough to influence the course of pneumonia is often associated with severe eye disturbances. Also the animal experiments[2] demonstrated that its effective curing dose and its lethal dose are too close together to allow a sufficient margin of safety. It is well known that substances containing an alcoholic hydroxyl group are often less toxic than the corresponding hydrocarbon residues. Because of the demonstrated value of optochin it seemed desirable to attempt the detoxification of this substance by the introduction of an hydroxyl in the ethyl ether group. This was accomplished by alkylating hydrocupreine with β-chloroethyl vinyl ether,[7] and subsequent hydrolysis of the vinyloxyethyl hydrocupreine to β-hydroxyethylhydrocupreine. This substance was also prepared by alkylation of hydrocupreine with ethylene chlorohydrin, and with glycol mono-*p*-toluene sulfonate, a very unstable reagent which must be used immediately after preparation. β-Hydroxyethylhydrocupreine had *in vitro* only one-fourth the pneumococcicidal power of optochin but proved to be of great interest because of its low toxicity and high protective power. Some data, furnished by the Medical Staff connected with the problem, on the pneumococcicidal activity and toxicity of this substance are given in Table I. The benzoyl derivative was prepared by benzoylation of hydroxyethylhydrocupreine and by alkylation of hydrocupreine with benzoylglycol *p*-toluenesulfonate.

Other modifications in the ethyl group of optochin yielded substances of such low bactericidal power as to make them unsuitable for our purpose. Chloroethylhydrocupreine was made by a method already described.[8] Vinylhydrocupreine was prepared from this substance by reaction with sodium ethoxide. Ethoxyethylhydrocupreine was synthesized using hydrocupreine and ethoxyethyl *p*-toluenesulfonate as alkylating agent. None of these preparations showed much efficiency in sterilizing pneumococcus cultures.

TABLE I

PNEUMOCOCCICIDAL ACTIVITY AND TOXICITY OF HYDROXYETHYLHYDROCUPREINE

	Quinine	Ethylhydrocupreine	Hydroxyethylhydrocupreine
In vitro. Sterilizes in concn of.	>1:50,000	1:800,000	1:200,000
Toxicity. Mice. Doses 5 mg.[a] Deaths	14/30	25/30	3/30
Protection. Mice. Doses 2 mg.[a] Recoveries	5/30	25/30	25/30

[a] Per 20 g. of body weight.

Further discussion of compounds used in this research, which have been described previously, has been almost entirely omitted for the sake of brevity. The work is being continued and other derivatives in the cinchona group will be described in future papers.

Experimental Part

Hydroxyethylhydrocupreine.—1.84 g. of sodium (0.08 mole) was dissolved in 100 cc. of absolute alcohol and 25 g. of hydrocupreine[9] (0.08 mole) was dissolved in the warm solution; 8.4 cc. of freshly distilled chloroethyl vinyl ether[7] was added and the solution heated in a sealed tube for twenty-two hours at 92–94°. The cold reaction product was poured into 200 cc. of dilute hydrochloric acid (containing 30 cc. of concd. hydrochloric acid) and the aqueous solution extracted twice with ether. The base was precipitated by slow addition of 10% sodium hydroxide in a large separatory funnel and extracted twice with ether. The ethereal solution was washed with water and extracted with dilute hydrochloric acid. This process was repeated twice. A residue insoluble in both alkali and ether was discarded. After the third precipitation the gummy base was washed by decantation and allowed to dry. It was dissolved in absolute alcohol and 2 *N* alcoholic hydrogen chloride was added until the solution was acid to Congo red. The solution was allowed to stand in the cooler until crystallization was complete and was then filtered. The mother liquor was evaporated under reduced pressure to somewhat less than half the original volume and an additional amount of hydrochloride was obtained. The air-dried salt weighed 9.3 g. It was dissolved in water and the base was precipitated with dilute ammonia, yield 6.3 g. The dried base was crystallized

(7) Cretcher and Pittenger, THIS JOURNAL, **46**, 1503 (1924).

(8) Slotta and Behnisch, *Ber.*, **66**, 360 (1933).

(9) Heidelberger and Jacobs, THIS JOURNAL, **41**, 817 (1919).

three times from dry acetone at 0° with some difficulty: yield 3.3 g. of crystals melting at 165° after preliminary softening between 105–112°, $[\alpha]_D$ −132.1° in absolute alcohol, $l = 1$, $c = 1$.

The crystallized base was reconverted to dihydrochloride and crystallized twice from absolute alcohol containing a little ether as white needles melting with decomposition at 234°, $[\alpha]_D$ −128.5° in absolute alcohol, $[\alpha]_D$ −177° in water, $l = 1$, $c = 1$.

Anal. Calcd. for $C_{21}H_{30}O_3N_2Cl_2$: C, 58.7; H, 7.1; N, 6.5; Cl, 16 5. Found: C, 58.6; H, 7.1; N, 6.4; Cl, 16.4.

Ethylene Glycol Mono-*p*-toluenesulfonate.—26 cc. of glycol (0.3 mole) was treated with 19 g. of *p*-toluene sulfonyl chloride (0.1 mole) in the presence of pyridine (16 cc.). After standing for thirty minutes, the mixture was treated with a large volume of water. The aqueous layer was separated from a heavy oil, which was filtered, dissolved in ether, washed several times with water and dried. Further purification did not appear to be practical as this substance changed quite rapidly on standing, depositing crystals of glycol di-*p*-toluenesulfonate.

Attempts to prepare glycol mono-*p*-toluenesulfonate under other conditions always yielded bis-ethylene-*p*-toluenesulfonate as white plates, recrystallized from alcohol, melting at 126°.

Anal. Calcd. for $C_{16}H_{18}O_6S_2$: S, 17.3. Found: S, 17.4.

Hydroxyethylhydrocupreine was prepared in small yield by alkylation with the partially purified glycol mono-*p*-toluenesulfonate described above, and also by alkylation with ethylene chlorohydrin. The alkaloid was isolated as dihydrochloride of specific rotation $[\alpha]_D$ −177°, in water, $l = 1$, $c = 1$.

Anal. Calcd. for $C_{21}H_{30}O_3N_2Cl_2$: Cl, 16.5. Found: Cl, 16.0.

Benzo-oxyethylhydrocupreine.—The benzoylation was carried out according to the method of Wunsch.[10] The base could not be obtained crystalline. It was therefore converted to neutral sulfate in alcoholic solution using litmus as indicator, and this salt was recrystallized from a mixture of alcohol and ether as white needles melting with decomposition at 231°, $[\alpha]_D$ −105.4° in alcohol, $l = 1$, $c = 1$.

Anal. Calcd. for $C_{28}H_{32}O_4N_2 \cdot 0.5H_2SO_4$: N, 5.5; S, 3.1. Found: N, 5.4; S, 3.3.

Benzoylglycol *p*-Toluenesulfonate.—Sixty-four grams of glycol monobenzoate[11] in 30 cc. of pyridine was treated with 38 g. of *p*-toluenesulfonyl chloride. The reaction mixture was left in the ice-box overnight and then treated with a large volume of water. The crystalline benzoylglycol *p*-toluenesulfonate was filtered off and washed with water and with ether. After recrystallization from methyl alcohol, the product melted at 74–75°; yield, 32 g.

Anal. Calcd. for $C_{16}H_{16}SO_5$: S, 10.0. Found: S, 10.0.

The position of the acyl group in benzo-oxyethylhydrocupreine was checked by an alkylation experiment with hydrocupreine and benzoylglycol *p*-toluenesulfonate. The reaction product was isolated as neutral sulfate; white needles melting with decomposition at 228°, $[\alpha]_D$ −103.4° in alcohol, $l = 1$, $c = 1$.

Chloroethylhydrocupreine.—This substance was prepared as described by Slotta and Behnisch;[8] yield 45% of the theoretical, $[\alpha]_D$ −125.1° in absolute alcohol; for the dihydrochloride $[\alpha]_D$ −163.3° in water, $l = 1$, $c = 1$.

We found no evidence of the gummy hydroxyethylhydrocupreine previously suggested[8] as forming in alkylation mixtures which were heated for eight hours.

Vinylhydrocupreine.—11.2 g. of β-chloroethylhydrocupreine dihydrochloride (0.025 mole) was added to 60 cc. of absolute alcohol containing 2.0 g. of sodium (0.085 mole). The solution, filtered from sodium chloride, was heated in a sealed tube for ten hours at 96°. After removal of the alcohol *in vacuo*, the residue was dissolved in ether and the ethereal solution was washed with water in a separatory funnel until the water was no longer alkaline. The base recovered from ether was converted to dihydrochloride in alcoholic solution. After several crystallizations from a 1:2 alcohol–ether mixture the salt had a constant rotation of $[\alpha]_D$ −174°, in water, $l = 1$, $c = 1$; yield, 4 g.

Anal. Calcd. for $C_{21}H_{26}O_2N_2 \cdot 2HCl$: Cl, 17.2; N, 6.8. Found: Cl, 17.0; N, 6.6.

A second very soluble dihydrochloride fraction of $[\alpha]_D$ about −150° was found in the mother liquors from the vinylhydrocupreine dihydrochloride. Not enough of this material was available to permit recrystallization to constant rotation.

Ethoxyethyltoluene Sulfonate.—Forty-five grams (0.25 mole) of *p*-toluenesulfonyl chloride was added in portions to a solution of 54 g. (0.6 mole) of glycol monoethyl ether in 80 cc. of dry pyridine. The solution was cooled to about 40°. After an hour a large volume of water was added and the heavy oil which settled out was washed repeatedly in a separatory funnel. The oil was taken up in ether and the solution was dried over sodium sulfate. After evaporation of the ether the product was distilled under reduced pressure. As the distillate had an acid reaction, it was washed with dilute sodium bicarbonate solution, then with water and finally dried; boiling point 186–187° at 3 mm., yield 27 g.

Anal. Calcd. for $C_{11}H_{16}O_4S$: S, 13.1. Found: S, 13.3.

Ethoxyethylhydrocupreine.—Six grams of hydrocupreine was alkylated with 5 g. of ethoxyethyl *p*-toluenesulfonate using conditions similar to those described by Slotta and Behnisch[8] for the preparation of chloroethylhydrocupreine. Neither the base nor the dihydrochloride of ethoxyethylhydrocupreine could be obtained in crystalline condition. The dihydrochloride was obtained as a uniform white powder after several precipitations from alcoholic solution with a large volume of ether; yield, 1.8 g., $[\alpha]_D$ −149°, $l = 1$, $c = 1$.

Anal. Calcd. for $C_{23}H_{32}O_3N_2 \cdot 2HCl$: Cl, 15.5. Found: Cl, 15.2.

Summary

A preliminary examination of the pneumococcicidal activity of a number of naturally occurring cinchona alkaloids, their hydrogenated derivatives and some artificially prepared alkaloids, has been made.

(10) Wunsch, *Ann. chim. phys.*, [VII] **7**, 125 (1896).

(11) Cretcher and Pittenger, THIS JOURNAL, **47**, 2560 (1925).

Hydroxyethylhydrocupreine is very interesting in this connection since it proved to be far less toxic to mice than optochin, and was highly efficient in protecting them against pneumococcic infection.

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The Reactivity of Nuclear Chlorine in Certain 5-Substituted Derivatives of 2-Chlorophenylarsonic Acid

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The Ullmann reaction, as applied in the field of organic arsenicals to date, has revealed that the halogen atoms in 2-chlorophenylarsonic acid,[2] 3-nitro-4-halogenophenylarsonic acids[3] and 2-chloro-5-nitrophenylarsonic acid[4] are quite reactive.

It was of interest to study the reactivity of the halogen in 2-chloro-5-carboxyphenylarsonic acid. This acid was readily condensed in an anhydrous alkaline medium in the presence of cuprous iodide, with ethanolamine, glycine and phenol. A comparison of reaction times and yields revealed that the reactivity of the chlorine atom in 2-chloro-5-carboxyphenylarsonic acid was the same as that of 2-chlorophenylarsonic acid. Incidental to the preparation of 2-chloro-5-carboxyphenylarsonic acid, 2-chloro-5-cyanophenylarsonic acid was isolated and identified. Condensations with this acid are more difficult to carry out due to tarry formations; however, it was condensed with phenol to form 2-arsono-4-cyanophenyl ether.

The effect of an ortho–para directing group substituted in the para position to the halogen in 2-chlorophenylarsonic acid was also studied, employing the 2-chloro-5-aminophenylarsonic acid and its acetyl derivative, as well as 2-chloro-5-hydroxyphenylarsonic acid and its carbethoxy derivative. Attempts to condense *p*-chlorophenol and various primary amines with these arsonic acids were unsuccessful.

This investigation has resulted in the preparation of several new organo arsenicals, one of which, 3-arsono-4-chlorobenzenediazonium chloride, in its general type is new to the field as an isolated and identified compound.

Experimental

2-Chloro-5-acetylaminophenylarsonic Acid.—To 5 g. of 2-chloro-5-aminophenylarsonic acid[5] dissolved in 20 cc. of *N* sodium hydroxide was added 10 cc. of acetic anhydride. When stirred with a glass rod, an instantaneous reaction occurred with the generation of much heat, and a white pasty mass resulted. To this solid was added 15 cc. of water and 6 *N* hydrochloric acid until the liquid was acid to Congo red paper, whereupon 2-chloro-5-acetylaminophenylarsonic acid separated as a mass of crystals; m. p. 225–227°. The needles rapidly absorbed one molecule of water of hydration, which they lost with effervescence at 160°.

2,2′-Dichloro-5,5′-diaminoarsenobenzene.—A mixture of 1.5 g. of 2-chloro-5-aminophenylarsonic acid, 5 cc. of 12 *N* hydrochloric acid and 30 cc. of water was heated until solution resulted and then 8 cc. of 50% hypophosphorous acid was added. The solution was heated at 60° for two hours. Upon the addition of sodium hydroxide until an alkaline reaction with Congo red paper was obtained, a light yellow, granular solid separated.

3-Arsono-4-chlorobenzenediazonium Chloride.—A mixture of 3 g. of 2-chloro-5-aminophenylarsonic acid and 3.5 cc. of 4 *N* hydrochloric acid was stirred into a white paste and placed in an ice–salt mixture. Nitrogen trioxide was passed into the paste until a clear dark green colored solution resulted. When this liquid was poured into a chilled mixture of 50 cc. of ethanol and 50 cc. of ether with stirring, the diazonium chloride separated as slender needles and was dried in a desiccator over sodium hydroxide sticks. The salt intumesced at 140°. It gradually assumed a red color upon standing in air, and heating increased this decomposition. It rapidly took up two molecules of water of hydration upon exposure to the atmosphere. When the diazonium chloride was refluxed with methyl alcohol, *o*-chlorophenylarsonic acid was obtained in a 70% yield.

2-Chloro-5-hydroxyphenylarsonic Acid.—A solution of 5 g. of 2-chloro-5-aminophenylarsonic acid in 50 cc. of water and 4 cc. of concentrated hydrochloric acid was diazotized with normal sodium nitrite solution until starch–iodide paper indicated an excess of nitrous acid. The resulting light red solution was heated on a water-bath at 70° until the evolution of nitrogen had ceased, water being added from time to time in order to maintain the original volume. The liquid was then divided into two parts and each evaporated to dryness on a water-bath in a 400-cc. beaker. The solid was dried completely in the oven at 110°, and the phenol extracted with anhydrous methyl alcohol. The methyl alcohol was evaporated on a water-bath and the red phenol extracted with *n*-butyl alcohol to remove any remaining inorganic salts. Upon the addition of

(1) Parke, Davis and Company Fellow.
(2) Etzelmiller, THIS JOURNAL, **53**, 3085 (1931).
(3) Maclay, *ibid.*, **54**, 3310 (1932).
(4) Hall, *ibid.*, **56**, 1779 (1934).
(5) Boehringer and Soehne, German Patent 286,547.