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

Cinchona Alkaloids in Pneumonia. X. Some Ethers of 6′-(β-Thiolethyl)-apocupreine

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A special interest attaches to thiols and their derivatives since such compounds often possess pronounced physiological[1] and therapeutic[2] properties.

We have therefore instituted a study of thiol derivatives of the cinchona alkaloids and now report on the preparation and properties of a number of ethers of 6′-(β-thiolethyl)-apocupreine having the general formula

$$R—S—(CH_2)_2—O—Q—CHOH—Qn=CHCH_3$$

where R = alkyl or aryl group, Q = quinoline residue, and Qn = quinuclidine residue. The thioethers were prepared by action of the appropriate potassium alkyl (or aryl) mercaptide on 6′-(β-chloroethyl)-apocupreine in boiling absolute ethanol.

Discussion of Results

Some of the properties of the bases and their dihydrochlorides are given in Table II, from which it may be seen that, as the length of the aliphatic side-chain at position 6′ is increased, the melting point and specific rotation of the bases get lower, although the molecular rotations remain approximately constant. In addition, it was found that with each increase in length of the side-chain there was an increase of solubility of the resulting free base in certain organic solvents.

The dihydrochlorides were examined for their toxicity to mice and their action against the pneumococcus *in vitro*, with the results shown in Table I. The progressive change in solubilities of the bases, on passing from the methyl to the *n*-butyl thioether, is accompanied by a corresponding increase in both bacteriostatic activity and toxicity (to mice) of the dihydrochlorides. The solubilities of the methyl and phenyl thioethers are very similar; the median lethal doses of their dihydrochlorides are practically equal, but the phenyl derivative has greater bacteriostatic power.

Owing to the high toxicity (compared with that of 6′-(β-hydroxyethyl)-apocupreine) of all the thioethers described here, protective action versus the pneumococcus *in vivo* was only ascertained for two of the compounds, the ethyl and the benzyl derivatives. In both cases oral administration of 10-mg. doses at zero, twenty-four, forty-eight, and seventy-two hours after infection (of mice) showed no protective action. However, the protective action of 6′-(β-chloroethyl)-apocupreine dihydrochloride was found to be practically identical with that of 6′-ethyl-apocupreine dihydrochloride.[3]

Experimental

β-Chloroethyl-*p*-Toluenesulfonate.—This compound was prepared essentially by the method of Clemo and coworkers.[4] The following properties appear to be new: boiling point, about 140° (1.5 mm.) (bath temp., 170–175°) and $n^{25}D$ 1.5280. On standing in the refrigerator the ester sets to a completely solid mass of rectangular-shaped crystals having m. p. +22.5°.

Anal. Calcd. for $C_9H_{11}O_3ClS$: Cl, 15.11; S, 13.67. Found: Cl, 15.12; S, 13.58.

Apocupreine.—Apocupreine acid sulfate was recrystallized from absolute methanol until the solubility remained constant at about 35.7 g. per liter of boiling absolute methanol (a minimum of two recrystallizations). It consisted of colorless crystals which developed a rich canary-yellow color on drying at 110° and then had $[\alpha]^{25}D$ −223° (in water, c = 1).

The acid sulfate was now reconverted to the free base, essentially by the method of Cretcher, *et al.*,[5] since that of Henry and Solomon[6] is not adaptable to large quantities. Sodium hydroxide solution (101 cc. of 8 *N*) was added to a suspension of 100 g. of recrystallized apocupreine acid sulfate in 1 liter of water. The resulting solution was diluted with 1 liter of water and the free base liberated by passing in carbon dioxide until precipitation was complete (*p*H about 8). Excess carbon dioxide must be avoided, since some of the alkaloid then redissolves. After washing the product with water and drying at 110° the yield of colorless, amorphous powder was the theoretical. It is readily crystallized by dissolving 10 g. in 30 cc. of cold pyridine and adding 50 cc. of water, or from 3 volumes of 95% ethyl alcohol. After thorough drying at room temperature it contains 1.5 moles water of crystallization. One mole of

(1) Weber, *Ber.*, **33**, 779 (1900); Riemann, *Protoplasma*, **10**, 82 (1930); Grandjean-Hirter, *Deut. med. Wochschr.*, **42**, 1316 (1916).

(2) Schoeller, *et al.*, U. S. Patent 1,685,341; Bourne, *J. Pharmacol.*, **28**, 409 (1926); Carpmael, British Patent 293,363; Sutton, *J. Am. Med. Assoc.*, **104**, 2168 (1935); Hansen and Fosdick, THIS JOURNAL, **55**, 2872 (1933).

(3) Butler, Renfrew, Cretcher and Souther, *ibid.*, **59**, 227 (1937).

(4) Clemo and Perkin, *J. Chem. Soc.*, **121**, 644 (1922); Clemo and Tenniswood, *ibid.*, 2549 (1931).

(5) Cretcher, Butler and Renfrew, U. S. Patent 2,033,515.

(6) Henry and Solomon, *J. Chem. Soc.*, 1923 (1934).

TABLE I

BACTERIOSTATIC ACTIVITY AND TOXICITY OF SALTS OF APOCUPREINE ETHERS[a]

Apocupreine	Bacteriostasis (*in vitro versus* Pnc. II in broth) is caused by concn. of	Intraperitoneal toxicity: Deaths, at dosages in mg. per 20 g. of mouse 1	2	3	4	5	6	Oral toxicity 20 mg.	30 mg.
Acid sulfate	>1: 50,000				2/30	13/30	27/30		
Dihydrochloride of									
Ethyl	1:800,000				21/130	94/130	78/85		
β-Chloroethyl	1:800,000			1/30	5/30	21/30	10/10		
Methyl-thioethyl	1: 50,000		0/10	5/30	16/30	8/10	5/5		
Ethyl-thioethyl	1:100,000			1/30	22/30		5/5		
n-Propyl-thioethyl	1:200,000		3/30	24/30					
n-Butyl-thioethyl	1:400,000	14/30	15/30	24/30	24/30	26/30	5/5		
Phenyl-thioethyl	1:400,000			3/30	19/30	28/30	10/10		
Benzyl-thioethyl	1:400,000		3/30	24/30	29/30	10/10		1/10	6/10

[a] These results were obtained by Drs. Bracken, Patrick, Corrado, Bream, Maclachlan and Johnston at the Mercy Hospital, Pittsburgh, Penna.

water is lost on drying at 110° (20 mm.) for sixteen to twenty hours. The remaining half mole is retained quite tenaciously and can only be removed by drying at 140° (20 mm.) during twenty-four hours.

Anal. Calcd. for $C_{19}H_{22}O_2N_2 \cdot 1.5H_2O$: $1H_2O$, 5.34. Found: H_2O (110°), 5.50. Calcd. for $C_{19}H_{22}O_2N_2 \cdot 1.5H_2O$: $1.5H_2O$, 8.01. Found: H_2O (140°), 7.92.

6′-(β-Chloroethyl)-apocupreine.—This was prepared as previously described[7] except that, in order to be able consistently to induce the dihydrochloride to crystallize, the following modifications were found advisable. After removal of potassium *p*-toluenesulfonate by filtration and of all ethyl alcohol under diminished pressure, the product was dissolved in chloroform (instead of ether), washed with alkali and water, dried and evaporated to dryness. This crude base was dissolved in acetone (10 volumes), and dry ether was added as long as brown flocculent impurity was precipitated. The impurity was filtered off and the product in the filtrate transformed to the dihydrochloride. This was dissolved in water, extracted twice with ether (to remove unchanged β-chloroethyl tosylate) and then five times with chloroform. (a) The *chloroform extracts* were united, evaporated to dryness, dissolved in absolute ethanol and reconverted to the dihydrochloride[8] which then readily crystallized from absolute ethanol (2 volumes). (b) The *aqueous solution* was evaporated to dryness to remove excess hydrochloric acid, dissolved in water, and treated as above, omitting the ether extraction. These procedures, (a) and (b), were repeated at least five times. The crystalline dihydrochloride was recrystallized, first from absolute ethanol (2 volumes) by adding dry ether (2 volumes), and then from absolute ethanol (2 volumes). It was reconverted to the free base, which had $[\alpha]^{22}D$ −179.5° (in absolute ethanol, c = 1). *Anal.* Calcd. for $C_{21}H_{25}O_2N_2Cl$: N, 7.5; Cl, 9.51. Found: N, 7.2; Cl, 9.46. Crystallized from aqueous pyridine it had m. p. 168° (with decomp.).

(7) Butler and Renfrew, THIS JOURNAL, **60**, 1473 (1938).

(8) Judging from the amount of hydrochloric acid required, the chloroform extracts contain the monohydrochloride.

General Method for Preparation of the Thio-ethers.—Potassium hydroxide (1.8 g. of 85%) was dissolved in 20 cc. of absolute ethanol and one proportion of the mercaptan was added. This solution was added to 10 g. of pure, dry β-chloroethyl apocupreine (regenerated from its twice-recrystallized dihydrochloride) and rinsed in with two 10-cc. portions of absolute ethanol. The solution was then boiled (hot water-bath), under a reflux condenser (soda-lime tube), during two hours. The precipitated potassium chloride was filtered from the cooled suspension (yield, quantitative). The alcohol was removed under diminished pressure, and the product dissolved in chloroform plus water. The aqueous layer was extracted twice with chloroform and then discarded. The chloroform extracts were united, washed with water, dried and evaporated to dryness. (The methyl-, phenyl-, and benzyl-thioethers crystallized out during the evaporation of the chloroform solution.) The yield of crude product was, in every case, practically quantitative.

The treatment from this stage on depended on the nature of the product. In some cases it could be crystallized directly. In other cases it was necessary to transform it to the dihydrochloride, remove traces of free mercaptan by extraction of the aqueous solution with ether, and then reconvert to the free base. Some of the properties and analyses of these thioethers are recorded in Table II.

Crystallization and Properties of the Bases.—Each of the thioethers was isolated as colorless crystals. Nucleating crystals of the *alkyl*-thioethyl ethers were obtained from a solution of the substance in dry ether; for the *aryl* ethers, chloroform was satisfactory. They were recrystallized as follows:

Methyl.—From absolute ethanol (10 g. in 20 cc.). It also crystallized from acetone, benzene, or heptane. *Anal.* Calcd. for $C_{22}H_{28}O_2N_2S$: C, 68.70; H, 7.34. Found: C, 68.63; H, 7.55.

Ethyl.—From dry ether (10 g. in 500 cc.). It also crystallized from absolute methanol or ethanol.

***n*-Propyl.**—From dry ether (10 g. in 500 cc.) or from ethanol-heptane.

TABLE II

MELTING POINTS, SPECIFIC ROTATIONS AND ANALYSES OF SOME APOCUPREINE ETHERS

Apocupreine ether, -thioethyl	Bases							Dihydrochlorides						
				Analyses, %					Analyses, %					
				Calculated		Found			Calculated			Found		
	M. p., °C.	Sp. rot.[a]	Formula	N	S	N	S	Sp. rot.[b]	N	S	Cl	N	S	Cl
Methyl-	155	−175°	$C_{22}H_{28}O_2N_2S$	7.29	8.3	7.39	7.8	−220°	6.13	7.0	15.51	6.14	6.9	15.86
Ethyl-	144–5	−172°	$C_{23}H_{30}O_2N_2S$	7.03	8.0	6.99	7.6	−213°[c]	[d]	6.3	13.98	[d]	6.4	13.72
n-Propyl-	147–8	−165°	$C_{24}H_{32}O_2N_2S$	6.80	7.8	6.83	8.0	−201° −176°[a]	5.77	6.6	14.61	5.50	6.0	14.01
n-Butyl-	141–2 120–1	−153°	$C_{25}H_{34}O_2N_2S$	6.57	7.5	6.44	7.7	−182°	5.61	6.4	14.20	5.37	5.6	14.43
Phenyl-	150–1	−149°	$C_{27}H_{30}O_2N_2S$	6.28	7.2	6.19	7.5	−168°[a]	5.40	6.2	13.66	5.11	6.4	13.12
Benzyl-	101–2	−133°	$C_{28}H_{32}O_2N_2S$	6.09	7.0	6.00	7.0	−162°	5.25	6.0	13.30	4.86	5.9	13.02

[a] 1% solution in absolute ethanol. [b] 1% solution in water. [c] Calculated for anhydrous substance (−198° for substance containing $2H_2O$). [d] For substance containing $2H_2O$.

***n*-Butyl.**—From dry ether, by spontaneous evaporation, m. p. 141–142°; or by dissolving 1 g. in 150 cc. of heptane containing 0.5 cc. of absolute ethanol, nucleating and standing overnight in the refrigerator, m. p. 120–121°. The two forms had the same analysis, for example:

Anal. Calcd. for $C_{25}H_{34}O_2N_2S$: C, 70.36; H, 8.04. Found: C, 70.24; H, 8.52.

Phenyl.—From absolute ethanol (10 g. in 50 cc.).

Benzyl.—From chloroform (10 g. in 20 cc.).

The methyl-thioethyl ether was sparingly soluble in cold but fairly soluble in hot dry ether or carbon tetrachloride; very sparingly soluble in cold and only sparingly soluble in hot pentane; and very sparingly soluble in cold but fairly soluble in hot heptane. On passing to the ethyl, *n*-propyl- and *n*-butyl-thioethyl ethers there was a graded increase in solubility in each of these four solvents. The *n*-butyl derivative was readily soluble in cold or hot dry ether or carbon tetrachloride; fairly soluble in cold or hot pentane; and sparingly soluble in cold but readily soluble in hot heptane. The solubilities of the phenyl and benzyl derivatives in these solvents resembled those of the methyl- and *n*-propyl derivatives, respectively.

Crystallization and Properties of the Dihydrochlorides.—The dihydrochlorides were prepared in the usual manner and were crystallized as follows:

Methyl.—Amorphous material (5 g.) was dissolved in 5 cc. of absolute ethanol and 2.5 cc. of dry ether was added, giving an almost solid mass of crystals. It was recrystallized from the same proportion of absolute ethanol without the addition of ether. It is readily soluble in cold water, giving a clear, colorless solution having a slightly bitter taste.

Ethyl.—Amorphous material (5 g.) was dissolved in 3 cc. of cold absolute ethanol. On standing overnight at room temperature the thick sirup had set to an almost solid mass of colorless crystals. Since this dihydrochloride is rather difficult to recrystallize, the most satisfactory method evolved so far is described. It was dissolved in one-third its weight of absolute ethanol, nucleated and allowed to crystallize at room temperature; dry ether was then added until the medium consisted of about 80% dry ether. After standing overnight in the refrigerator there was obtained a suspension of crystals which could be filtered readily. After drying in the vacuum desiccator it contained 2 molecules of water of crystallization. It dissolves in water to a clear, colorless solution having a slightly bitter taste.

Anal. Calcd. for $C_{23}H_{30}O_2N_2S \cdot 2HCl \cdot 2H_2O$: H_2O, 7.10. Found: H_2O, 7.01.

Benzyl.—It was recrystallized from absolute ethanol (5 g. in 20 cc.). It is soluble in water to give a faintly opalescent solution which is practically devoid of taste.

We were unable to isolate the following dihydrochlorides in crystalline form: ***n*-propyl- and *n*-butyl-.** They are soluble in water to give very faintly opalescent solutions having a somewhat bitter taste. **Phenyl.**—It is not completely soluble in water at its own *p*H but dissolves in 0.1 *N* hydrochloric acid (1 g. in 10 cc.) giving a colorless, practically tasteless solution.

Summary

Some ethers of 6′-(β-thiolethyl)-apocupreine have been prepared and their chemical and pharmacological properties ascertained. Although they exhibit bacteriostatic power *versus* the pneumococcus they are too toxic to be of practical value.

6′-(β-Chloroethyl)-apocupreine dihydrochloride is found to have *in vitro* pneumococcicidal power, toxicity to mice and *in vivo* protective action against the pneumococcus (in mice) which are practically identical with those of 6′-ethylapocupreine dihydrochloride.

PITTSBURGH, PA. RECEIVED FEBRUARY 11, 1942