A Quinidine Transformation Product Isolated from Quinidine Injection

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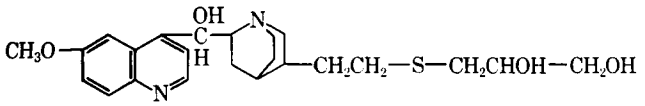
Abstract □ A substance found in quinidine gluconate injection containing α-thioglycerol as a stabilizer has been identified as α-(6 - methoxy - 4 - quinolyl) - 5 - (5,6 - dihydroxy - 3 - thia - *n*-hexyl)-2-quinuclidine methanol. It is a product of anti-Markownikoff addition of thioglycerol to the vinyl group of quinidine.

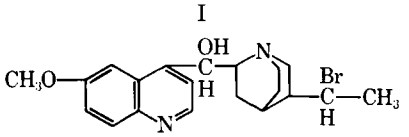
Keyphrases □ Quinidine gluconate injection—transformation product □ Degradation product, quinidine—isolated, identified □ Column chromatography—separation □ IR spectrophotometry—identity □ Crystallographic properties—identity

It has been reported from this laboratory (1) that samples of quinidine gluconate injection, USP, in which α-thioglycerol was present as a preservative contained a significant quantity of a substance which, while possessing many of the characteristics of quinidine, differed from it in several essential features. The substance was separated from quinidine by chloroform extraction of the quinidine as its ion-pair from *p*-toluenesulfonic acid solution. The substance, which remained in the aqueous solution, was then extracted as the free base after the solution was made alkaline. The IR spectrum was closely similar to that of quinidine; the only essential difference was stronger hydroxyl absorbance, consonant with the partition behavior of the material.

The substance has been identified as α-(6-methoxy-4-quinolyl)-5-(5,6-dihydroxy-3-thia-*n*-hexyl)-2-quinuclidine methanol (I), the product of anti-Markownikoff addition of thioglycerol to the vinyl group of quinidine. The injection from which the product was isolated is formulated with 1% of α-thioglycerol as a stabilizer.



I



II

After extraction as previously described (1), the adduct was readily crystallized as the free base from a tetrahydrofuran-benzene mixture.

To prepare larger amounts of the adduct, an aqueous solution of quinidine sulfate was allowed to stand at room temperature with an excess of α-thioglycerol; conversion of the quinidine occurred in high yield. The adduct so obtained was identical with that isolated from the commercial injection.

The elemental analysis of the product is in agreement with that of a 1:1 molar adduct. Assignment of anti-Markownikoff addition is based upon NMR data; it is consistent with the report of Smith and Hernestam (2) that addition of mercaptans to double bonds is exclusively anti-Markownikoff. The principal diagnostic feature of the NMR spectrum is the absence of the terminal methyl group which would result from Markownikoff addition. The corresponding methyl group of α-bromodihydroquinidine (II), formed by Markownikoff hydrobromination of quinidine (3), is readily discerned, appearing as a doublet at 1.72 p.p.m. downfield from tetramethylsilane; the same methyl group of dihydroquinidine produces a triplet at 0.85 p.p.m. Based upon these values and upon the relative electron-withdrawing properties of bromine and sulfur, it would be expected that the methyl group of Markownikoff quinidine-thioglycerol adduct would produce a doublet in the region of 1.0 to 1.5 p.p.m. The spectrum of the actual adduct does not evidence absorbance with any definition in the region between 0 and 2 p.p.m., where absorbance representing the methylene groups of the quinuclidine ring appears.

EXPERIMENTAL

Dihydroquinidine was prepared by hydrogenation of an ethanolic solution of quinidine sulfate over platinum oxide at a pressure of 50 p.s.i., according to Heidelberger and Jacobs (4). α-Bromo-dihydroquinidine was prepared by heating quinidine sulfate in 48% HBr at 40–50° for 3 days, following the procedure of Gibbs and Henry (3).

Isolation of Adduct from Commercial Injection—The adduct was separated from intact quinidine as previously described (1). The chloroform solution of the base was concentrated and the base precipitated by addition of benzene. The base was redissolved in a small volume of tetrahydrofuran; upon addition of benzene it separated as well-defined crystals. After drying *in vacuo* over refluxing xylene for 1 hr., they melted at 176–178°. Examination by differential thermal analysis showed the product to be free of solvent. The principal refractive index were: α, 1.565; β, 1.605; γ, 1.713.

Synthesis of Adduct—Quinidine sulfate (20 g.) was suspended in 150 ml. of water, and dilute sulfuric acid was added dropwise to bring it into solution. Then 20 ml. of 90% α-thioglycerol was added, and the mixture was diluted to 250 ml. The solution was allowed to stand in the dark for 2 weeks at room temperature.

To 20-ml. portions of the solution was added 20 ml. of 40% *p*-toluenesulfonic acid solution; the major portion of the nonreacted quinidine was extracted with six 25-ml. portions of chloroform and the chloroform extracts were discarded. The solution was made alkaline by careful addition of solid sodium carbonate and the liberated adduct extracted with chloroform. The extracts were passed over chromatographic columns prepared with 8 g. of diatomaceous earth[1] and 6 ml. of 10% *p*-toluenesulfonic acid solution, and the columns were washed with an additional 75 ml. of chloroform to remove the last amounts of quinidine. The columns were extruded and the adduct was recovered as before (1); 15 g. of crude adduct was recovered.

To purify the material, 2 g. was dissolved in 75 ml. of tetrahydrofuran and an equal volume of benzene was added. The optical-

[1] Celite 545, Johns-Manville Co.

crystallographic properties of the resultant crystals were the same as those of the product isolated from the commercial injection, and the X-ray diffraction patterns of the material from the two sources were identical.

Anal.—Calcd. for $C_{23}H_{32}N_2O_4S$: C, 63.86%; H, 7.46%; N, 6.48%; S, 7.41%. Found: C, 64.00%; H, 7.24%; N, 6.34%; S, 7.49%.

Preparation of the Hydrochloride—Five grams of the adduct was dissolved in 20 ml. of isopropanol, and the calculated volume of 1 *N* alcoholic hydrochloric acid to form the monohydrochloride was added. After 50 ml. of acetone was added, the solution was allowed to stand in the refrigerator; 4.73 g. of the monohydrochloride was recovered. Recrystallization from isopropanol yielded crystals (m.p. 120–124°) containing one molecule of isopropanol of crystallization. Calculated for B·HCl·1-iPrOH: 11.36% isopropanol; found (loss of weight on heating at 110° under vacuum): 11.1%.

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In Vitro Binding of Salicylates to Saliva Proteins

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Abstract □ A method has been developed whereby the *in vitro* binding of salicylic acid, acetylsalicylic acid, and salicylamide to saliva proteins, may be easily determined. It was found, using gel filtration, that salicylates added to saliva are bound to the saliva proteins to the extent of from 35 to 50% after a contact time of from 0.5 to 2.0 hr. at 37°.

Keyphrases □ Salicylate binding—saliva proteins □ Saliva proteins—ninhydrin test □ Fluorometry—analysis

The salicylate drugs have been studied continuously for many years, but several important questions regarding the *in vivo* distribution and the metabolic fate of these drugs have remained unanswered. One such question deals with the degree to which the salicylates bind to the proteins in various body fluids, especially the saliva.

Many methods have been reported to demonstrate the various aspects of drug-protein interaction, but the literature is void with respect to interactions between saliva proteins and the following salicylate drugs: acetylsalicylic acid, salicylic acid, and salicylamide. Goldstein (1), in a review, stated that most of the methods could be classified into three groups: methods based upon the reduction in the free drug concentration, the alteration of the drug properties, and the alteration of the protein properties.

Klotz (2), and Davison and Smith (3), have employed dialysis to demonstrate drug-protein interactions. The latter investigators used such a procedure to show the binding of salicylic acid and related substances to purified samples of protein material.

The development of gel filtration methods between 1959 and 1964 (4–7) made possible the column separation of materials of different molecular weight. These methods were adapted to drug-protein binding studies (8–9), some of which allowed the estimation of the comparative magnitude of drug-protein binding. Potter and Guy (10) developed a spectrofluorometric method for the determination of plasma salicylate content. A dextran gel filtration procedure, rather than a solvent extraction or a protein precipitation procedure, was used for the separation of the bound from the unbound salicylate. This method also permitted the rapid estimation of the magnitude of the binding. Sturman and Smith (11) have recently expanded plasma studies to include the fractionation, by a gel filtration procedure, of protein-bound salicylate drugs from the plasma of various species of animals.

The above information shows that gel filtration procedures will adequately separate bound from unbound salicylate drugs. It has also been shown, by Lange and Bell (12), that the submicrogram concentrations of the salicylates that would be expected to be present in the saliva, can be determined by fluorometric means. This paper describes the utilization of gel filtration and fluorometry for the *in vitro* determination of the degree of salicylate-protein interaction that takes place in human saliva.

EXPERIMENTAL

Reagents and Chemicals—The reagents and chemicals used were: acetylsalicylic acid powder USP,[1] salicylic acid crystals USP,[2] sodium hydroxide USP,[2] sodium phosphate (dibasic) granular,[2] salicylamide powder,[3] synthetic polysaccharide,[4] and naphthol blue-black.[5] Ferric chloride solution, T.S., and 0.2% aqueous ninhydrin solution were used as indicators.

Apparatus—A synthetic polysaccharide (Sephadex K-25) column having an internal diameter of 25 mm. was used for the gel filtration procedure. A universal fraction collector (G.M.), equipped with a microflex timer, was used to mechanize the collection of the eluent fractions. Analyses were made using fluorometer (Turner

[1] Mallinckrodt Chemical Works.
[2] S. B. Penick and Co.
[3] New York Quinine and Chemical Works.
[4] Sephadex G-25, Coarse, Pharmacia Fine Chemicals, Inc.
[5] Allied Chemical Co.