[CONTRIBUTION FROM THE CHEMISTRY SECTION LABORATORY, IOWA STATE COLLEGE]

THE REPLACEMENT OF SECONDARY HYDROXYL GROUPS BY SULFONIC ACID SUBSTITUENTS[1]

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Interest in a precise method for the synthesis of steroid sulfonic acids was created when it was found that a monosulfonic acid derivative of cholesterol was antirachitic (1). The acid was formed by a high temperature sulfonation of the sterol which produced a preponderance of other substances from which a brucine salt could be isolated in crystalline form. The sulfonic acid group appears to be on a secondary carbon and no more satisfactory method was available for the synthesis of such sulfonic acids.

There are published procedures (2, 3) for the preparation of alkyl sulfonic acids from primary alcohols or alkyl halides and from secondary and tertiary alkyl halides (4, 5) from their Grignard reagents but these are not successful with steroids. Ketosulfonic acids of steroids (6, 7) are readily made but the carbonyl group limits their use. A generally useful synthesis of alkyl sulfonic acids by the conversion of secondary hydroxyl groups to the corresponding sulfonic acid substituent can now be reported.

A reaction of cholesteryl halide (Ia) with thiourea, analogous with that of alkyl halides with thiourea (3, 8) was found by Rosenberg and Turnbull (9) to form cholesterylisothiouronium hydrohalide (IIa) from which they produced thiocholesterol by alkali hydrolysis. However a more practical procedure for the preparation of cholesterylisothiouronium salts as the *p*-toluenesulfonates (II) was described by King, Dodson, and Subluskey (10) and perfected by Ralls, Dodson, and Riegel (11).

It appears that no one has succeeded in converting steroid isothiouronium derivatives (II) into sulfonic acids even though thiocholesterol can be produced readily from the isothiouronium salts. The conversion of thiocholesterol to the 3-β-sulfonic acid is probably prevented because the first product of the known methods of oxidation is the disulfide (10) which if not insoluble requires treatment too drastic to cleave properly. A procedure has now been perfected in which in one step a variety of alkyl isothiouronium *p*-toluenesulfonates can be converted to alkyl sulfonic acids. The sulfonated carbon may be secondary and the procedure succeeds with one double bond in the alkyl group by first protecting the bond by bromination then debrominating with sodium iodide according to the procedure of Schoenheimer (12). Moreover the dibromo derivative may be debrominated with quinoline to produce a doubly unsaturated sulfonic acid. Formation of insoluble disulfides is prevented through the formation of performic acid by the addition of hydrogen peroxide to the formic acid solution of the isothiouronium salts (13, 14). Oxidation proceeds smoothly to the sulfonic

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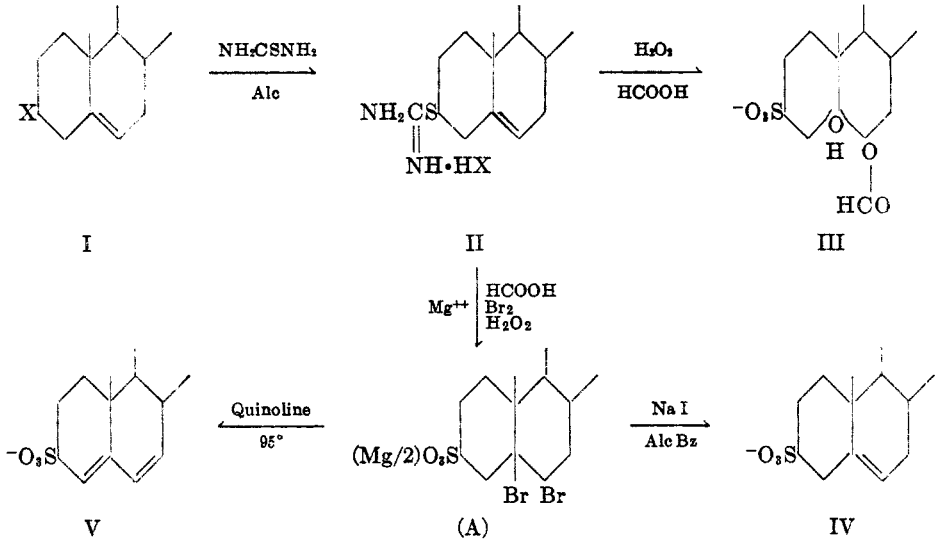
acid which can be precipitated by dilute aqueous solutions of polyvalent cations and filtered off. To prevent interference with a similar sulfonic acid in subsequent purification in case the hydrocarbon group is small like that in the *p*-toluenesulfonic acid which is also present, the latter may be precipitated first as the sodium salt with sodium methoxide in methanol, filtered from the isothiouronium base and replaced with formic acid.



X *p*-Toluenesulfonyl—
a X = Cl— or Br—
b Brucine salt

Purification of the sulfonic acids was accomplished through the formation of their brucine salts by a procedure perfected recently (1) in the isolation of an antirachitic cholestatetraene sulfonic acid. These salts crystallize nicely from hot distillation residues of their chloroform solutions on dilution with hot acetone or methyl ethyl ketone in which they are quite insoluble.

There is no apparent reason why primary hydroxyl groups could not be equally well converted to the sulfonic acid substituent. Difficulty was encountered in the conversion of the hydroxyl group in alcohols like ergosterol which have more than one double bond to protect from oxidation by the peracid. This report details preparations of such sulfonic acids which have heretofore been unobtainable and presents the scope of a procedure for the conversion of the secondary hydroxyl group to the corresponding sulfonic acid substituent.

Further investigations of this type of sulfonation of more highly unsaturated steroid derivatives are under way.

Acknowledgment. Credit and appreciation are due Mr. Donald Trimnell of our section for the determinations of the ultraviolet absorption spectra.

EXPERIMENTAL PART

I. Brucine 5-hydroxy-6-formoxycholestane-3β-sulfonate (IIIb). Cholesterylisothiouronium *p*-toluenesulfonate, 2.5 g. (0.004 mole) made according to the procedure of Ralls, *et al.*

(11) was warmed to solution in 40 ml. of 98% formic acid in a hood. After cooling to 30°, 4 ml. of 30% hydrogen peroxide was stirred in dropwise with a thermometer and the temperature was kept below 35° by a cooling bath until the suspension cleared and no more spontaneous warming took place. The solution then was warmed to 50° in a water-bath for one hr. A small amount of insoluble matter was separated on a suction filter and 20 ml. of water and 0.3 g. of magnesium oxide powder were stirred into the filtrate. The mixture was evaporated with a water pump and the residue was triturated with 15 ml. of water and collected on a suction filter, washed with 5 ml. more water, then with 15 ml. of acetone. The crude magnesium salt amounted to 1.9 g. (92%). It was further purified and crystallized by conversion to the brucine salt as follows. The cake was treated with a 15 ml. solution of 1.5 g. of brucine in chloroform and transferred to a separatory-funnel with a 10% solution of acetic acid. The chloroform phase was twice washed with 20-ml. portions of the dilute acetic acid then once with water and distilled at 55° to a thick residue with a water pump. The residue in methylene chloride again was vacuum-distilled and the hot residue was dissolved in 15 ml. of boiling methyl ethyl ketone. The brucine sulfonate crystallized on cooling and weighed 2.6 g; m.p. 255–256° $[\alpha]_D^{25}$ in $CHCl_3$, −19.5°.

Anal. Calc'd for $C_{23}H_{26}N_2O_4 \cdot C_{28}H_{48}O_6S$: N, 3.09; S, 3.53.
Found: N, 3.16; S, 3.48.

II. Brucine Δ⁵-cholestene-3β-sulfonate (IVb). Cholesterylisothiouronium *p*-toluenesulfonate (6.3 g., 0.01 mole) was triturated in a 200-ml. beaker to a fine suspension in 50 ml. of 98% formic acid. While stirring under a hood a solution of 0.5 ml. (1.1 moles) of bromine in 20 ml. of formic acid was added dropwise. Solution took place. Then 6 ml. of 30% hydrogen peroxide was stirred in and the temperature maintained at 35–40° by a cooling bath as necessary for one hour. The solution soon clouded up as the 5,6-dibromosulfonic acid separated to produce a thick mass. It was next heated in a bath at 50° for 20 min., then cooled and mixed with 100 ml. of water and 0.8 g. of magnesium oxide powder. The magnesium salt was collected on a suction filter and the cake was triturated with 30 ml. of 0.5% magnesium acetate solution and pressed down again on the filter. The cake was triturated again, this time with 70 ml. of acetone and again it was collected and left to air dry to 6.2 g. (A).

To debrominate, the dibromo magnesium sulfonate (A) was refluxed one hr. with 3 g. of sodium iodide in 75 ml. of ethanol and 50 ml. of benzene. The red mixture was decolorized with the minimum of a saturated sodium sulfite solution and was distilled with a water pump until free of solvent. The residue was mixed with a 25 ml. solution of 4.5 g. of brucine in chloroform and transferred to a separatory-funnel with 10% acetic acid. After shaking, the chloroform phase was again washed with 10% acetic acid then once with water, transferred to a distilling flask, and distilled to 10 or 15 ml. with a water pump from a 60°-bath. Addition of 25 or 30 ml. of hot methyl ethyl ketone and cooling caused crystallization of the brucine sulfonate in clumps of colorless needles. The filtrates from the first crops, concentrated to solidification and diluted with more acetone, gave further crystals of the salt and a total of 5 g., m.p. 199–200°; $[\alpha]_D^{25}$ in chlorofrom, −20.8°.

Anal. Calc'd for $C_{23}H_{26}N_2O_4 \cdot C_{27}H_{36}O_3S$: N, 3.31; S, 3.79.
Found: N, 3.26; S, 3.76.

(*a*) The brucine Δ⁵-cholestene-3β-sulfonate was readily converted through its sodium salt to the calcium salt. The brucine salt, one g., well dispersed in 20 ml. of water, was stirred with a solution of 0.15 g. of sodium hydroxide pellets in 40 ml. of water. The mixture deposited brucine as the sodium salt formed in solution. The alkaloid, collected on a suction filter, washed with water, and vacuum-dried, weighed 0.5 g. The filtrate and washings were neutralized with acetic acid and then treated with excess saturated calcium acetate solution. The precipitated calcium salt was centrifuged out, washed with water, then with acetone and vacuum-dried to give 0.306 g. at 60° as the calcium salt.

Perbenzoic acid titration: 0.303 g. used 0.418 cc. of 0.1 *N* or 0.97 mole equivalent.

The ultraviolet absorption spectra of a solution of the calcium Δ⁵-cholestone-3β-sulfonate, in methanol, gave no maxima between 320 and 220 mμ.

III. Brucine Δ⁴,⁶-cholestadiene-3β-sulfonate (Vb). Crude magnesium 5,6-dibromocholes-

tane-3β-sulfonate (A) above, 2.4 g., was treated with 15 to 20 ml. of chloroform and 1.2 ml. of quinoline and transferred to a separatory-funnel with 10% acetic acid. The chloroform phase was twice shaken out with 10% acetic acid and once with water then transferred to a distilling flask and distilled under the water pump until free of chloroform. The residue in 15 ml. more quinoline then was heated at 95° in a water-bath under the vacuum of a water pump for one hr. The residue in 15 ml. of a chloroform solution of 2 g. of brucine was transferred to a separatory-funnel with 10% acetic acid and washed, concentrated, and crystallized from hot acetone as before. The yield of crude brucine $\Delta^{4,6}$-cholestadiene-3β-sulfonate was 1.9 g. To remove traces of quinoline the brucine salt was converted to the calcium salt as in Procedure II (*a*), then back to recrystallization as the brucine $\Delta^{4,6}$-cholestadiene-3β-sulfonate as in Procedure I. The yield was 1.7 g., m.p. 207–209°; $[\alpha]_D^{25}$ in chloroform, −21.3°.

Anal. Calc'd for $C_{23}H_{26}N_2O_4 \cdot C_{27}H_{44}O_3S$: N, 3.34; S, 3.80.
Found: N, 3.23; S, 3.67.

Perbenzoic acid titration: 0.24 g. of the calcium salt used 0.908 ml. of 0.1 *N* or 2.2 mole equivalents.

The ultraviolet absorption spectra of a solution of the calcium salt in methanol, gave maxima at 290, 240, and 233 mμ in contrast to no maxima in this region for calcium Δ^5-cholestene-3β-sulfonate. The peak at 240 mμ almost coincides with a maximum at 242 mμ for $\Delta^{4,6}$-cholestadienol-3 in cyclohexane found by Schaltegger (15).

IV. D-*Bornylisothiouronium p-toluenesulfonate.* The D-borneol was tosylated according to a modified procedure of Freudenberg and Hess (16). A solution of 5 g. of D-borneol in 15 ml. of dry pyridine was treated with 9 g. (1.5 moles) of *p*-toluenesulfonyl chloride which had been recrystallized from hexane. After standing overnight the mixture was extracted with ether and a slight excess of 10% hydrochloric acid, washed in a separatory-funnel with water, dried with sodium bicarbonate, and vacuum-distilled. The residue of 11 g. crystallized on cooling. Without purification 12.5 g. (0.04 mole) of this ester was refluxed in 70 ml. of isopropyl alcohol with 6.2 g. (0.08 mole) of thiourea for 12 hrs. The solvent was removed by vacuum-distillation from a 60°-bath. The partly crystalline residue was triturated with water and collected on a filter. The cake then was triturated in 30 ml. of acetone, filtered off, and air-dried to 11 g. Recrystallized from isopropyl alcohol, it melted at 178°.

Anal. Calc'd for $C_{18}H_{28}N_2O_3S_2$: N, 7.28; S, 16.68.
Found: N, 7.31; S, 16.85.

V. Brucine D-*bornyl-2-sulfonate.* D-Bornylisothiouronium *p*-toluenesulfonate (2 g., 0.005 mole) in 20 ml. of ether, cooled in an ice-salt bath, was treated with similarly cooled 5.2 ml. of 1.01 *N* sodium methoxide in methanol. The precipitated sodium *p*-toluenesulfonate was separated in a Gooch crucible fitted to a neck of a two-neck distillation flask under water suction and the filtrate was collected in 5 ml. of formic acid in 15 ml. of ether within the flask. After washing the Gooch filter with ether the solvent was removed by vacuum-distillation to dryness from a bath at 50°. The residue of D-bornylisothiouronium formate was dissolved in 15 ml. of 98 % formic acid, cooled to 20° in the ice-bath, and treated with 2.5 ml. of 30% hydrogen peroxide while stirring with a thermometer. The temperature was permitted to rise to 35° and when no further spontaneous heating occurred the solution was heated in a bath at 50° for 20 min. Then 0.3 g. of magnesium acetate in 10 ml. of water and 1 ml. of methanol were added and the solution was evaporated with the water vacuum at 50° until all of the magnesium sulfonate had separated. This was collected on a suction filter and after washing with acetone it was air-dried to 1.7 g. It was purified as the brucine salt by treating with 3 g. of brucine in chloroform in a beaker and transferring to a separatory-funnel with 10% acetic acid for the usual washing, distillation of the chloroform and crystallization from hot methyl ethyl ketone. This brucine salt recrystallized nicely from the boiling ketone with enough boiling benzene to dissolve it; m.p. 275–280° with decomposition; $[\alpha]_D^{25}$ in chloroform −18.0°.

Anal. Calc'd for $C_{23}H_{26}N_2O_4 \cdot C_{10}H_{18}O_3S$: N, 4.57; S, 5.23.
Found: N, 4.76; S, 5.03.

VI. 3-Heptylisothiouronium p-toluenesulfonate. This derivative was prepared according to procedure IV. A 20 hour reflux with thiourea was necessary, however. Trituration of the isopropyl alcohol distillation residue with isopropyl ether, filtration, and washing the cake with water left the crude isothiouronium salt. The tosyl ester, 9.6 g., gave 6.5 g. of 3-heptylisothiouronium *p*-toluenesulfonate on recrystallization from isopropyl alcohol and water, m.p. 130°.

Anal. Calc'd for $C_8H_{18}N_2S \cdot C\ H_8O_3S$: N, 8.08; S, 18.51.
Found: N, 8.05; S, 18.57.

VII. Brucine heptane-3-sulfonate. This product was prepared proportionately the same as in procedure V and by starting with 3.5 g. of 3-heptylisothiouronium *p*-toluenesulfonate. The crude magnesium sulfonate, 0.9 g., was isolated and converted as in procedure V to 1.6 g. of the brucine heptane-3-sulfonate, m.p. 245–246°; $[\alpha]_D^{25}$ −22.3° in chloroform.

Anal. Calc'd for $C_7H_{16}O_3S \cdot C_{23}H_2N_2O_4$: N, 4.87; S, 5.58.
Found: N, 4.64; S, 5.65.

SUMMARY

1. Procedures are described for the two step conversion of secondary alcohols from their crude *p*-toluenesulfonate esters through their thiocarbamated derivatives to sulfonic acids.

2. Hydrogen peroxide in formic acid oxidized the alkylisothiouronium *p*-toluenesulfonates in formic acid solution to the alkyl sulfonic acids.

3. All sulfonic acids were purified and crystallized through their brucine salts.

4. Unsaturated groups in alcohols such as cholesterol were (a) preserved against oxidation by bromination followed by debromination with sodium iodide or (b) increased by dehydrobromination with quinoline.

5. The general scope of the procedure was demonstrated by the preparation of the new crystallized salts,— brucine 5-hydroxy-6-formoxycholestane-3β-, $\Delta^{5,6}$-cholestene-3β-, $\Delta^{4,6}$-cholestadiene-3β-, bornyl-2-, and heptyl-3-sulfonates.

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