Synthesis of Salicylamide Sulfate and its Effect on Calcium Ion Solubility

By WILLIAM O. FOYE, RONALD N. DUVALL, MONTGOMERY H. TALBOT, and ROBERT H. ZAIM

Salicylamide sulfate has been synthesized and found to be chromatographically identical to a metabolic product appearing in the urine of patients on salicylamide therapy. This compound, when incubated with suspensions of calcium hydroxide, was found to increase the aqueous solubility of calcium ion beyond that expected from a salt effect alone. An explanation of the effect of salicylamide in halting calcium stone growth has been suggested.

SALICYLAMIDE SULFATE (I), commonly referred to as an "ethereal" sulfate but actually a sulfate ester, has been postulated as a metabolite of salicylamide in humans. Litter, *et al.* (1), claimed that 36% of the drug was excreted by humans as the sulfate without giving experimental details, and Becher, *et al.* (2), showed that a metabolite of salicylamide from humans liberated sulfate after hydrolysis. Foye, *et al.* (3), also hydrolyzed the urines collected from stone-formers and found that a metabolite, supposed to be the sulfate according to Becher's procedure, liberated both salicylamide and sulfate in the hydrolysate. Quantitative determinations on the urines of a large number of stone-forming patients gave an average "ethereal" sulfate excretion of 31% of the salicylamide ingested. Mandel, *et al.* (4), did not detect the sulfate among the metabolites of salicylamide from three cancer patients, but possibly their countercurrent extraction procedure was too lengthy to obtain the sulfate unhydrolyzed.

Salicylamide sulfate has not previously been isolated. To identify positively that the sulfate measured quantitatively in human urines was salicylamide sulfate, the compound has now been synthesized and shown to be chromatographically identical with the urinary product. Although the substance did not migrate with the solvent systems used, the faintly flourescent spot it gave on paper chromatograms did not appear in the urines of the patients in the absence of salicylamide ingestion. A supply of this compound was also required to determine the extent of any solubilization it might exert on calcium ion, since salicylamide ingestion has been found to halt calcium stone growth in a number of stone-formers (5).

The procedure of Burkhardt (6) for preparing the sulfate of phenol using dimethylaniline and potassium pyrosulfate was attempted for the synthesis of salicylamide sulfate, but the major product was dimethylaniline-*m*-sulfonic acid. Baumann's procedure (7) for the preparation of salicylic acid sulfate (no per cent yield was mentioned), however, was adapted to the present synthesis, and the potassium salt of salicylamide sulfate was obtained in low yield. The low yield is not surprising when it is considered that the compound was prepared in an aqueous medium and is readily hydrolyzable. The compound was found to be quite hygroscopic and unstable when exposed to air for any length of time, which probably accounts for the discrepancy in the analytical value for carbon. The product was chromatographically homogeneous, however, and showed no evidence of contamination with salicylamide or other salicylic acid derivatives. Attempts to prepare salicylamide sulfate under nonaqueous conditions were unsuccessful.

Solubility determinations were made in water for calcium hydroxide in the presence of salicylamide sulfate, as well as with sodium chloride, measurements being made up to 3 hours, when hydrolysis of the salicylamide sulfate becomes noticeable. A definite increase in the solubility of calcium hydroxide was noted beyond that to be expected from the salt effect alone (see Table I). Examination of the data in Table I reveals an apparent increase with time in the solubility of calcium hydroxide when in the presence of salicylamide sulfate. This could be due to slow formation of a calcium ion salicylamide sulfate complex, but is more likely the result of experimental variation or error. The same may be said for the apparent decrease in solubility of calcium hydroxide in water or in the presence of sodium chloride.

It appears likely that a so-called weak salt or complex is formed between salicylamide sulfate and

TABLE I.—SOLUBILITY OF CALCIUM ION IN THE PRESENCE OF SALICYLAMIDE SULFATE AND AN INDIFFERENT SALT

Sample	Substance Added	Time, min.	$[Ca^{++}]$, m*M*/ml.
1	0.04 *M* SS[a]	40	0.0410
2	0.04 *M* SS[a]	105	0.0411
2	0.04 *M* SS[a]	145	0.0423
4	0.04 *M* SS[a]	185	0.0433
1	0.04 *M* NaCl	40	0.0287
2	0.04 *M* NaCl	105	0.0263
3	0.04 *M* NaCl	145	0.0240
4	0.04 *M* NaCl	185	0.0266
1	0.03 *M* SS	40	0.0262
2	0.03 *M* SS	105	0.0276
3	0.03 *M* SS	145	0.0292
4	0.03 *M* SS	185	0.0291
1	0.03 *M* NaCl	40	0.0204
2	0.03 *M* NaCl	105	0.0190
3	0.03 *M* NaCl	145	0.0177
4	0.03 *M* NaCl	185	0.0197
1	none	40	0.0186
2	none	105	0.0182
3	none	145	0.0168
4	none	185	0.0160

[a] SS, salicylamide sulfate.

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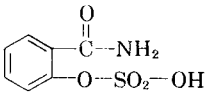
calcium ion, which could explain the halt in calcium stone growth observed in stone-formers when taking salicylamide daily. While the increase in solubilization is small [molar concentrations of salicylamide sulfate were used which approximate those to be expected in urines from the amount of salicylamide (2–3.6 Gm.) ingested by stone-formers on this therapy], it is sufficient to explain the failure of existing stones to accumulate the few mg. of calcium salts per day that ordinarily deposit in the kidneys of stone-forming patients.



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EXPERIMENTAL

Potassium Salicylamide Sulfate.—Salicylamide (20 Gm., 0.146 mole) was dissolved in 40 ml. of distilled water containing 16 Gm. (0.285 mole) of potassium hydroxide. Finely powdered potassium pyrosulfate (34 Gm., 0.134 mole), previously heated to 350–400°, was added slowly, with stirring. The mixture was stirred for 6 hours at a temperature of 60–70°, and the pH was maintained between 8 and 9 by the addition of saturated potassium hydroxide solution.

The mixture was then shaken with 50 ml. of 95% ethanol and filtered. After cooling, the mixture was treated with 150–200 ml. of ether, with shaking. The aqueous layer was separated and neutralized to pH 7 by the dropwise addition of glacial acetic acid. Precipitated salicylamide was removed, and the filtrate was evaporated to dryness by means of a current of dry air. Absolute ethanol (200 ml.) was added to the residue, and a white, powdery product was filtered, washed with absolute ethanol, and air-dried for a few hours before being stored in a desiccator. The yield was 1.42 Gm. of product which softened at 145–150° and melted at 220–230°.

Anal.[1]—Calcd. for $C_7H_6KNO_5S$: C, 32.93; H, 2.37; S, 12.56. Found: C, 31.70; H, 2.35; S, 12.76.

The compound is extremely soluble in water and insoluble in absolute ethanol. Its aqueous solution has a nearly neutral pH and gave a violet color with ferric chloride T.S. only after hydrolysis with dilute hydrochloric acid. It gave no precipitate with barium chloride solution and dilute hydrochloric acid until the solution was heated for a few minutes. The barium sulfate was isolated, weighed, and the percentage of sulfur calculated. The total sulfur present was thus shown to be sulfate ester.

The compound gave a positive flame test for potassium. It is unstable in air at room temperature. An aqueous solution showed the same faintly fluorescent spot on a paper chromatogram, which did not migrate in the solvent system used, 1-butanol saturated with 3 *N* ammonia, as was obtained in the urines of patients on salicylamide therapy, but not in the absence of salicylamide ingestion. Hydrolysis of the material liberated salicylamide and sulfate ion.

Solubility Determinations.—Test tubes containing 50 ml. of distilled water with excess calcium hydroxide, and either 50 ml. of salicylamide sulfate solution (the appropriate concentration of the potassium salt neutralized with hydrochloric acid), or 50 ml. of sodium chloride solution of the appropriate normality were mechanically stirred in a constant temperature bath at 37°. Aliquots, 2 ml., were withdrawn at the indicated times by means of a pipet whose tip was covered with filter paper. From each aliquot, 1 ml. was used to determine calcium ion by a modification of the method of Hawk, Oser, and Summerson (8) (the calcium oxalate was centrifuged rather than filtered, and was washed with 20% ammonia water instead of distilled water) and 1 ml. was used to determine inorganic sulfate. The latter determinations showed no significant hydrolysis of the ethereal sulfate during the course of the experiment. For longer periods of time, appreciable hydrolysis of the salicylamide sulfate was observed, which prevented the determination of solubility products under equilibrium conditions.

[1] Carbon-hydrogen analysis was done by Carol K. Fitz, Needham, Mass.

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Digitoxin Antagonism by Visnadin

By D. D. VARONOS, P. C. VOUKYDES, and G. C. NIKITOPOULOU

Visnadin, a lactone derived from bishop's weed, has been found to have marked antidotal properties in animals poisoned with digitoxin. It increases survival on acute and chronic administration of the glycoside, prevents the appearance of bradycardia, and reverses cardiac arrhythmias.

DIGITALIS glycosides probably have the smallest margin of safety of any of our commonly used drugs and they appear to have resisted all attempts at improvement in this respect by chemical modification. A less desirable alternative would be to have available a reliable antidote, but this has not yet been found. Recently, a naturally occurring lactone, visnadin (1), has been introduced in Europe for the treatment of the anginal syndrome (2) and has been shown by Smith, *et al.* (1), to have 10 times

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