[CONTRIBUTION FROM THE LABORATORY OF RADIOCHEMISTRY, DEPARTMENT OF CHEMISTRY, AND THE GASTRIC LABORATORY, DEPARTMENT OF INTERNAL MEDICINE, COLLEGE OF MEDICINE, UNIVERSITY OF CINCINNATI]

COMPOUNDS FOR CANCER RESEARCH. V. RADIOACTIVE SULFONAMIDES[1]

FRANCIS EARL RAY[2] AND LOUIS SOFFER[3]

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This paper describes the syntheses of a series of sulfonamides prepared in a continuation of the search by this laboratory for a substance which will localize selectively in tumor tissue (1, 2). Were such a material found and made radioactive it would have diagnostic and therapeutic potentialities.

The selection of the sulfonamide linkage as a fundamental structure was made for the following reasons: (a) the function is apparently not attacked by enzymes; (b) sulfonamides have been shown to reduce the effective vitamin intake of animals by suppressing the intestinal flora, and this reduction of essential vitamins in the diet of tumor-bearing mice is known to inhibit the growth of tumors (3); and (c) sulfonamides have been reported to localize in certain tissues (4).

Although no localization of a chemical material in malignant tissue is known to be involved, the following experiments are suggestive. Boyland (3) administered large and repeated doses of certain aromatic amines containing sulfur such as 4,4′-diaminophenyl sulfoxide and sulfamyl sulfanilic acid, and reported the inhibition of tumors in mice. With further investigation complete inhibition of growth of spontaneous tumors in 4 of 4 mice was obtained by methylene blue, 4,4′-diaminophenyl sulfoxide, and 4,4′-diaminophenyl ether, the last being generally most effective (5). In general the inhibition of tumor growth continued only for the period of dosing.

It has been shown in rats that tumor (hepatoma) glycolysis lowers the *p*H of the tumor from 7.0 to 6.4, primarily because of the formation of lactic acid (6). Therefore, an injection of glucose a short time prior to the ingestion of a sulfonamide might very conceivably, by lowering the *p*H in the tumor, suppress the ionization of an acidic sulfonamide, thus reducing its solubility and forcing its deposition in the cancer tissue.

A desirable compound would require that its solubility at *p*H 6.4 should be perhaps one half the solubility at the *p*H of blood serum, 7.4. Schmitt and colleagues (7) examined six sulfonamides and found for four of these that the ratio of the solubility at *p*H 7.2 to that at *p*H 6.4 was two or greater. With *p*H 7.4 as the upper limit these ratios were even more pronounced.

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[2] Present address, Cancer Research Laboratory, University of Florida, Gainesville, Florida.

[3] Present address, Department of Chemistry, Iowa State College, Ames, Iowa.

For dibasic acids Michaelis (8) has derived the following relationship between solubility and pH.

$$\Lambda = \lambda\left(1 + \frac{K_I}{[H^+]} + \frac{K_I K_{II}}{[H^+]^2}\right)$$

Λ = total solubility = concentration of all the material present, whether as the undissociated molecule or ion.
λ = partial solubility = concentration of the undissociated molecule in the saturated solution.
K_I = primary ionization constant.
K_{II} = secondary ionization constant.
$[H^+]$ = concentration of hydrogen ion.

We may now set up two equations for the solubility–pH relationship at the two pH values in question, 7.4 and 6.4. We may neglect the $\frac{K_I K_{II}}{[H^+]^2}$ term since it will be a very small number. Eliminating λ from the two equations by setting $\Lambda_{7.4} = 2\,\Lambda_{6.4}$, we obtain

$$2\left(1 + \frac{K_I}{[H^+]_{6.4}}\right) = 1 + \frac{K_I}{[H^+]_{7.4}}$$

Solving for K_I:

$$K_I = \frac{[H^+]_{7.4}[H^+]_{6.4}}{[H^+]_{6.4} - 2[H^+]_{7.4}} = \frac{(3.98 \times 10^{-8})(3.98 \times 10^{-7})}{(3.98 \times 10^{-7}) - 2(3.98 \times 10^{-8})}$$
$$= 4.98 \times 10^{-8}.$$

This is the minimum value of the ionization constant for our sulfonamide.

Work recently carried out in this laboratory has indicated that tumor mice show a considerably different uptake picture of radioactive iodosulfapyridine than normal mice (9). If this can be extended to man, it would be a possible means for the diagnosis of malignancy.

DISCUSSION AND PROCEDURE

It is proposed that the radioactivity be introduced as S^{35} in p-toluenesulfonyl chloride prepared from toluene and sulfuric-S^{35} acid.[4] It was obviously essential to prepare the p-toluene-S^{35}-sulfonyl chloride in the highest possible yield and purity. By the use of a water-removing apparatus, stirring, and excess toluene, the yield of sodium p-toluene sulfonate based on sulfuric-S^{35} acid was raised from 38% (10) to 60%. p-Toluenesulfonyl chloride could then be obtained in satisfactory yield and purity by heating sodium p-toluene-S^{35}-sulfonate with a mixture of phosphorus pentachloride and phosphorus oxychloride.

The mono- and di-tosylated derivatives and certain intermediates of the following aromatic amines were prepared: benzidine, 4,4′-diaminophenyl sulfide, 4,4′-diaminodiphenylmethane, and 4,4′-diaminophenyl ether.

[4] Obtained from the U. S. Atomic Energy Commission, Oak Ridge, Tennessee.

Monotosyl derivatives of benzidine and 4,4′-diaminodiphenylmethane were prepared by masking one amino group by acetylation. The monotosyl derivative of 4,4′-diaminophenyl sulfide was best obtained by the tosylation of 4-nitro-4′-aminophenyl sulfide, followed by reduction of the nitro group to the amine. The monotosyl derivative of 4,4′-diaminophenyl ether was prepared by condensing *p*-hydroxyacetanilide with *p*-bromonitrobenzene and sodium hydride in Methyl Carbitol solution.

EXPERIMENTAL

Sodium p-toluene-S^{35}-sulfonate. A three-necked, standard-taper flask equipped with a thermometer, a mercury-sealed stirrer, and a side-arm water trap attached to a condenser sealed against moisture, was assembled. The water trap was packed with calcium chloride-glass beads to 0.5 of an inch below the side arm. The trap was filled with purified toluene (11) until the liquid level was slightly below the level of calcium chloride. A mixture of 10 ml. (0.18 mole) of H_2SO_4 containing 4 mc. of $H_2S^{35}O_4$ and 20 ml. (0.19 mole) of toluene (11) was heated with stirring at such a rate that the temperature rose to 195° in one hour. The flame was removed, the solution cooled to 100°, and 3–4 ml. of toluene was added with thorough mixing. Heating was resumed. This process was repeated through four or five additions, with simultaneous removal of wet toluene from the bottom of the trap, until no more water (cloudiness) was seen on heating. The side arm was emptied partly and the flask heated to 150° to remove excess toluene. The total time required was five hours.

The hot reaction mixture was washed into 100 ml. of water. The solution was neutralized by the careful addition of sodium hydroxide, followed by 30 g. of sodium chloride. The mixture was heated to boiling, and water was added, if necessary, to dissolve the salt. Filtration removed 2–3 g. of yellow, waxy *p*-tolyl sulfone ($CH_3C_6H_4SO_2C_6H_4CH_3$). On cooling in an ice bath sodium *p*-toluene sulfonate separated and was collected. The cake was washed with 25 ml. of saturated sodium chloride solution and pressed dry.

After dissolving the cake in 50 ml. of water, 10 g. of sodium chloride was added, a further 20–25 ml. of water being required to bring the latter into solution. The solution was stirred a few minutes with 0.5 g. of charcoal, filtered hot, and concentrated to 70–75 ml. Crystallization was permitted to take place without disturbance in the cold. The yield of colorless material was 19.7–22 g. (56.6–63.2%) (Fieser reports 37.8%). The sulfonate was identified and its purity confirmed by preparation of the *p*-toluidine salt (m.p. 197°) (10). An attempt to increase the yield only led to increased sulfone formation.

p-Toluenesulfonyl-S^{35} chloride. A mixture of 10 g. (0.051 mole) of sodium *p*-toluene-S^{35}-sulfonate (dried at 140°), 5 g. (0.024 mole) of phosphorus pentachloride, and 10 ml. (0.109 mole) of phosphorus oxychloride was refluxed with frequent shaking at 160° for 1.5 hours. Excess phosphorus oxychloride was removed at the pump and the mass washed into 400 ml. of ice-water. The nearly white solid was washed with a little cold water and dried over phosphorus pentoxide. The material weighed 8.6 g. (89.6%) and melted at 69°. This is the literature value (19).

N,N′-Di-p-tosyl-S^{35}-benzidine was prepared from 6.1 g. of purified benzidine (13), 14.5 g. of *p*-toluene-S^{35}-sulfonyl chloride, and 20 ml. of dry pyridine. The dried material melted at 236–239° and weighed 9.4 g. Successive recrystallizations from aqueous acetone and charcoal gave a colorless product weighing 7.6 g. (40.6%). The large crystalline plates melted at 248°. Willstätter (12) reports 243°.

The radioactivity was measured using a Geiger counter 4 mg./sq. cm. and a Higginbotham scaler circuit. Comparison was made with a sample of the original $H_2S^{35}O_4$. A sample of two ml. containing 0.00107 g. gave 115 c./m. or a total of 813,960 c./m. The original sample of $H_2S^{35}O_4$ measured at the same time gave an equivalent of 2,550,000 c./m. The yield was therefore 31.9%. A less pure sample weighing 1.644 g. was obtained from the mother liquors which gave an equivalent of 188,000 c./m. or 7.39%. The mother liquors were examined and a total recovery in all steps was 79% based on the original $H_2S^{35}O_4$. Further

investigation will be necessary to determine the sources of error but it was probably due to the different forms in which the S^{35} was measured.

The following experiments were carried out with non-radioactive material but the methods were exactly those suitable for employment with our radioactive *p*-toluene-S^{35}-sulfonyl chloride.

N-Acetylbenzidine was prepared by the procedure of Cain (14). Recrystallization from 50% alcohol and charcoal yielded a colorless material melting at 199–200°. Cain (14) reports m.p. 199°.

N-Acetyl-N'-p-tosylbenzidine was prepared by stirring for one hour on the water-bath a solution of 2.26 g. (0.01 mole) of N-acetylbenzidine, 1.91 g. (0.01 mole) of *p*-toluenesulfonyl chloride, and 10 ml. of pyridine. The color quickly changed from purple-red to orange. The solution was added slowly and with stirring to 125 ml. of ice-water, made acidic with concentrated hydrochloric acid, and left for several hours. The lightly colored solid when filtered weighed 3.1 g.; m.p. 218–221°. Recrystallization from aqueous alcohol and charcoal, followed by drying at 120–130°, yielded 2.8 g. (73.8%) of colorless microcrystalline needles melting at 227.5°.

Anal. Calc'd for $C_{21}H_{20}N_2O_3S$: S, 8.43. Found: S, 8.37, 8.25.

N-p-Tosylbenzidine. To a hot solution of 1.0 g. (0.0026 mole) of N-acetyl-N'-*p*-tosylbenzidine in 25 ml. alcohol was added 25 ml. of 20% hydrochloric acid. The mixture was heated at reflux for 40 minutes. The solution was concentrated at the pump with warming to remove alcohol, cooled, and neutralized with concentrated ammonia with stirring. The yield was 0.9 g. of lightly-colored material melting at 158–164°. Recrystallization from aqueous alcohol and charcoal afforded 0.8 g. (91%) of near-colorless crystalline material, m.p. 164–165°. It was previously prepared from *p*-tosylhydrazobenzene or azobenzene and *p*-toluenesulfinic acid (20).

Anal. Calc'd for $C_{19}H_{18}N_2O_2S$: S, 9.47. Found: S, 9.0, 8.95.

N-N'-bis(p-Tosylaminophenyl) sulfide. A solution of 2.16 g. (0.01 mole) of *bis*(*p*-aminophenyl) sulfide (15), 3.82 g. (0.02 mole) of *p*-toluenesulfonyl chloride, and 15 ml. of dry pyridine was treated as previously described. The crude material melted at 184–189°. Several recrystallizations from absolute alcohol (charcoal) yielded 4.3 g. (82%) of colorless crystalline material melting sharply at 195°.

Anal. Calc'd for $C_{26}H_{24}N_2O_4S_3$: S, 18.33. Found: S, 18.40.

N-p-Tosylamino-p'-nitrophenyl sulfide. After this paper was prepared for publication an account of the preparation of this compound by Baker, Querry, and Kadish appeared (21). They reported m.p. 154–155°. Our material, after several recrystallizations from benzene-petroleum ether mixture, melted at 157.5–158.5° and weighed 3.2 g. (80%).

Anal. Calc'd for $C_{19}H_{16}N_2O_4S_2$: S, 16.01. Found: S, 15.9, 15.85.

N-p-Tosylamino-p'-aminophenyl sulfide. To a hot solution of 2.0 g. (0.005 mole) of N-p-tosylamino-p'-nitrophenyl sulfide in 20 ml. of glacial acetic acid was added, during one minute, 4.5 g. (0.02 mole) of stannous chloride dihydrate dissolved in 8 ml. of hot concentrated hydrochloric acid. After warming at 60° for 30 minutes the solution was cooled, an equal volume of benzene added, and the solution was neutralized by carefully adding concentrated ammonia with vigorous shaking. The benzene layer was removed, fresh benzene added, and the extraction repeated. The extract was dried over calcium sulfate, filtered through glass wool, and the benzene removed. The light-yellow solid was dissolved in 30% alcohol and permitted to stand overnight (seeding was at times necessary for crystallization to occur). Yield, 1.5 g. of material melting at 140–143°. Recrystallization from 30% alcohol (charcoal) gave 1.3 g. (70.4%) of colorless glistening microcrystals, m.p. 142–143°.

Anal. Calc'd for $C_{19}H_{18}N_2O_2S_2$: S, 17.30. Found: S, 17.12.

N,N'-di-p-Tosylaminodiphenylmethane. A solution of 3.96 g. (0.02 mole) of *p,p'*-diaminodiphenylmethane, 7.64 g. (0.04 mole) of *p*-toluenesulfonyl chloride, and 30 ml. of pyridine was warmed on the water-bath for one hour and poured with stirring into 300 ml. of cold water. Kuhn, Jacob, and Furter (22) using sodium hydroxide instead of pyridine reported m.p. 164°. Two recrystallizations of our material with aqueous alcohol (charcoal), followed by drying at 120°, gave 6.2 g. (61%) of colorless crystals, m.p. 186–187.5°.

Anal. Calc'd for $C_{27}H_{26}N_2O_4S_2$: S, 12.65. Found: S, 12.31.

N-p-Acetylamino-p'-aminodiphenylmethane. This compound was prepared according to the method of Kaslow and Stayner (23) who reported m.p. 135.5–136°. We obtained m.p. 133–134°.

Anal. Calc'd for $C_{15}H_{16}N_2O$: N, 11.66. Found: N, 11.38, 11.59.

N-p-Tosylamino-N'-p-acetylaminodiphenylmethane. A solution of 2.40 g. (0.01 mole) of N-*p*-acetylamino-*p'*-aminodiphenylmethane and 1.90 g. (0.01 mole) of *p*-toluenesulfonyl chloride in 10 ml. of pyridine was warmed for one hour on the water-bath and the solvent removed at the pump. The reddish oil remaining was taken up in aqueous alcohol and permitted to stand overnight. A sample of the dried material melted at 167–168°. Recrystallization from aqueous alcohol (charcoal) yielded 3.25 g. of micro needles, m.p. 167.9–169.2°. Yield, 82.5%.

Anal. Calc'd for $C_{22}H_{22}N_2O_3S$: N, 7.10. Found N, 7.15.

On two occasions in the preparation of this compound a material was obtained which melted at 136–137°. A mixed melting point determination showed no depression but instead, a sharply defined transformation at 137° with no further visible change until 169°, at which point there was complete melting. Possibly these were polymorphic forms; however, there was evidence, though apocryphal, of both quantitative and qualitative nature that the lower-melting substance was a pyridine adduct—that is, two molecules of pyridine had combined with one of the normal product. A lack of material prevented a complete elucidation of the matter.

N-p-Tosylamino-p'-aminodiphenylmethane. To a hot solution of 1.97 g. (0.005 mole) of N-*p*-tosylamino-N'-*p*-acetylaminodiphenylmethane in 20 ml. of alcohol was added 25 ml. of 20% hydrochloric acid and the mixture refluxed for 30 minutes. The solution was concentrated at the pump to remove alcohol and concentrated ammonia was added dropwise with stirring until the mixture was alkaline. After standing overnight the white precipitate was filtered, washed with water, and dried. The crude material melted at 137–140°. Recrystallization from aqueous alcohol (charcoal) yielded 1.4 g. (79%) of a near-white crystalline material, m.p. 156–158°.

Anal. Calc'd for $C_{20}H_{20}N_2O_2S$: S, 9.11. Found: S, 9.0, 9.1.

N,N'-di-p-Tosylaminophenyl ether. Ditosylation was carried out as previously described using 2 g. of *p,p'*-diaminophenyl ether and 3.82 g. of *p*-toluenesulfonyl chloride. Several recrystallizations from aqueous alcohol (charcoal) yielded a white crystalline substance weighing 3.6 g.; m.p. 179–180°. Yield, 71%.

Anal. Calc'd for $C_{26}H_{24}N_2S_2O_5$: S, 12.61. Found: S, 12.22, 12.41.

N-p-Acetylamino-p'-nitrophenyl ether. This compound has been prepared by Ravrick, Brewster, and Dains (24) from *p*-nitrofluorobenzene and *p*-hydroxyacetanilide. They reported m.p. 153°. We used *p*-hydroxyacetanilide, *p*-bromonitrobenzene, sodium hydride, copper powder, and Methyl Carbitol. The melting point was the same but the yield was somewhat less (28–32%).

Anal. Calc'd for $C_{14}H_{12}N_2O_4$: N, 10.3. Found: N, 10.0, 10.3.

p-Nitro-p'-aminophenyl ether. To a hot solution of 1.36 g. (0.005 mole) of N-*p*-acetylamino-*p'*-nitrophenyl ether in 15 ml. of alcohol was added 25 ml. of 20% hydrochloric acid and reflux maintained for 45 minutes. On standing the amine hydrochloride separated in light-yellow, feathery needles. These were washed with a small amount of cold water and dried at 120°; m.p. 215–217°, weight, 0.75 g.

Concentrated ammonia was added slowly with stirring to the filtrate and the yellow precipitate permitted to settle. After filtering, washing, and drying, the free base weighed 0.28 g., m.p. 128–129°. The total crude yield on the basis of the free amine was 87%. Recrystallization of the free base from aqueous alcohol (charcoal) afforded a yellow, finely crystalline material, m.p. 134–135°.

Anal. Calc'd for $C_{12}H_{10}N_2O_3$: N, 12.18. Found: N, 12.2, 12.1.

N-p-Tosylamino-p'-nitrophenylether. A solution of 1.33 g. (0.005 mole) of *p*-nitro-*p'*-aminophenyl ether hydrochloride and 0.95 g. (0.005 mole) of *p*-toluenesulfonyl chloride in 5 ml. of pyridine was warmed for one hour on the water-bath and poured into 200 ml. of

cold water. Concentrated hydrochloric acid was added with stirring until the mixture was definitely acidic. Recrystallization of the gray-yellow solid from alcohol (charcoal) yielded 1.45 g. (75.5%) of fine crystals, m.p. 154–155°.

Anal. Calc'd for $C_{19}H_{16}N_2O_5S$: N, 7.29. Found: N, 7.32.

N-Tosylamino-p'-aminodiphenyl ether. To a hot solution of 1.92 g. (0.005 mole) of N-*p*-tosylamino-*p'*-nitrophenyl ether in 20 ml. of glacial acetic acid was added, during one minute, 4.5 g. (0.02 mole) of stannous chloride dihydrate dissolved in 8 ml. of hot, concentrated hydrochloric acid. After warming at 60° for 30 minutes the solution was cooled and transferred to a separatory funnel. An equal volume of benzene was added and the solution neutralized by carefully adding concentrated ammonia with vigorous shaking. The benzene layer was removed, fresh benzene added, and the extraction repeated. After drying over calcium sulfate, the extract was filtered through glass wool and the benzene removed. Recrystallization of the red solid from aqueous alcohol (charcoal) yielded 1.1 g. (77.5%) of white microcrystalline needles, m.p. 141–142°.

Anal. Calc'd for $C_{19}H_{18}N_2O_3S$: N, 7.91. Found: N, 7.91, 7.84.

An alternate preparation of this material, but in poor yield, was obtained from *p,p'*-diaminophenyl ether dihydrochloride, *p*-toluenesulfonyl chloride, and pyridine.

SUMMARY

In continuation of the search for substances that will localize in tumor tissue the preparation of a series of sulfonamides and certain intermediates is described, together with the proposed scheme for making these materials radioactive. As an example, the preparation of N,N'-di-*p*-tosyl-S^{35}-benzidine is given in detail.

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CINCINNATI, OHIO

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