130. STUDIES IN DETOXICATION

II. THE IDENTIFICATION OF CATECHOL-4-SULPHONAMIDE AS A METABOLIC PRODUCT OF *p*-HYDROXY-BENZENESULPHONAMIDE IN THE RABBIT.

THE SYNTHESIS OF DERIVATIVES OF CATECHOLSULPHONAMIDE

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IN Part 10 of this series [Sammons *et al.* 1941] it was stated that when *p*-hydroxy-benzenesulphonamide (I) was fed to rabbits a small portion was oxidized to a catechol derivative, probably catechol-4-sulphonamide (1:2-dihydroxybenzene-4-sulphonamide, II). Several unsuccessful attempts had been made to isolate this compound. The reason for these failures became evident when attempts were made to synthesize it (see below) since it was found that synthetic catecholsulphonamide was a non-crystalline resinous substance. Furthermore, much difficulty was experienced in separating it from phenolsulphonamide in the urine extracts. Eventually it was found that the separation could be effected by acetylation, for whilst phenolsulphonamide was readily acetylated in aqueous solution with acetic anhydride and sodium carbonate in the cold, catecholsulphonamide was only partially acetylated. The acetylated phenolsulphonamide was insoluble and hence the main bulk of it could be removed leaving the partially acetylated catechol compound in the solution, from which it could be extracted with ether.

Since catecholsulphonamide was a resin, a search had to be made for a crystalline derivative whereby it could be characterized. Methylation of the synthetic resinous catecholsulphonamide or veratrole-4-sulphonamide with methyl sulphate and alkali gave crystalline *veratrole-4-sulphondimethylamide* (III) and this substance could be used for characterizing catecholsulphonamide. Veratrolesulphondimethylamide was obtained on suitable treatment of ether extracts of hydrolysed urine from phenolsulphonamide-fed rabbits, thus proving the presence of catechol-4-sulphonamide in the urine.

In Part 10 it was shown that about 80% of a 2 g. dose of phenolsulphonamide could be accounted for by the ethereal sulphate and glucuronide excreted. The yield of pure veratrolesulphondimethylamide was 250 mg. and, since 24 g. of

phenolsulphonamide were fed, about 1 % of the latter was oxidized to the catechol compound. Large losses, however, are inevitable in such experiments and it is thought that much more than 1 % is oxidized; a figure of 4-5 % would probably be more correct.

This work on phenolsulphonamide was carried out in order to gain experience for tackling the problem of the oxidation products of sulphanilamide, since in Part 8 of this series [Thorpe *et al.* 1941] some evidence was obtained that, in the rabbit at least, sulphanilamide undergoes a small amount of oxidation to an *o*-aminophenolsulphonamide in a manner similar to the oxidation of phenolsulphonamide to catecholsulphonamide.

The synthesis of catecholsulphonamide derivatives

The main initial difficulty in this synthesis was the preparation of catechol-4-sulphonic acid in good yield. Several attempts were made to use the directions for the preparation of this acid given in a German patent [cf. Friedländer, 1917] which consisted of heating sodium 1:2-dichlorobenzene-4-sulphonate with NaOH in the presence of copper in a sealed tube at 220°. The results, however, were unsatisfactory. An unsuccessful attempt was also made to use the fact discovered by Paul [1906, 2] that catecholsulphonic acid was produced when guaiacol-4- and -5-sulphonic acids (both obtained during sulphonation of guaiacol at 100°) are heated to 125°. A very satisfactory method was eventually obtained in which catechol was sulphonated directly at 0°. Cousin [1893] had shown that catechol in contact with pure sulphuric acid at 0° for several days was converted into a monosulphonic acid. Later, Gentsch [1910] showed that this acid was catechol-4-sulphonic acid and not the -3-sulphonic acid as had been previously thought, since on methylation it gave veratrole-4-sulphonic acid, a compound of known structure [Paul, 1906, 1]. The sulphonic acid (V) obtained in this way was acetylated with acetic anhydride to give the *pyridine salt* (VI)

which was easily converted into *1:2-diacetoxybenzene-4-sulphonyl chloride* (VII). From this chloride, sulphonamides of the type represented by VIII ($X = H$ or other group) could be made.

EXPERIMENTAL

Identification of catecholsulphonamide in urine after phenolsulphonamide. Phenolsulphonamide (2 g.) dissolved in warm water was administered (stomach tube) daily for 3 days to each of four rabbits. The urine was collected daily for 4 days following the first dose of the sulphonamide (see Table 1). Each batch of urine was hydrolysed by boiling for an hour with 1/5 vol. of conc. HCl. The hydrolysed urine was filtered and then extracted with ether in a large continuous extractor of 1 l. capacity. The ethereal extracts were combined and dried for a short time with anhydrous calcium chloride. After filtration, the solution was evaporated under diminished pressure leaving a blackish residue consisting of tarry material mixed with crystals. The residue was now dissolved in 400 ml. water and filtered to remove some black insoluble matter. The clear brownish

Table 1. *Extraction of sulphonamides from urine*

Day	No. of rabbits	Total phenol-sulphonamide fed g.	Urine vol. ml.	Extraction time hr.
1	4	8	—	—
2	4	8	650	8
3	4	8	800	20
4	4	0	580	20
5	4	0	500	20
Total 24				

filtrate, which gave an intense green colour with FeCl_3 , was treated with 20 g. anhydrous Na_2CO_3 and when the latter had dissolved, 16 ml. of acetic anhydride were added. The whole was thoroughly mixed by shaking and after a short time acetoxybenzenesulphonamide separated. After 1 hr. at 0° , the acetyl compound was filtered off and washed well with water. The combined filtrates (ca. 700 ml.) were slightly acid in reaction and gave a faint catechol FeCl_3 reaction. This reaction became very intense after heating the solution with a little dilute NaOH . The acetyl compound (13.4 g.) gave only a faint colour with FeCl_3 under these conditions, showing that nearly all the catechol compound was in the filtrates. The latter was now evaporated *in vacuo* at 50° and during this process a further 0.7 g. of acetoxybenzenesulphonamide was recovered. The residue from this evaporation was semi-solid and bulky and contained much sodium acetate derived from the acetylating reagents. It was therefore dissolved in 200 ml. water, made strongly acid with HCl and then extracted for 16 hr. with ether in a continuous extractor. The ethereal extract was evaporated leaving a clear brownish syrup, which gave an intense green colour with FeCl_3 and reduced ammoniacal AgNO_3 in the cold.

Methylation of the syrup and isolation of veratrolesulphondimethylamide. The whole of the above syrup, after a preliminary trial on a small sample, was methylated by stirring with 12 ml. methyl sulphate and 30 ml. of 10% NaOH , each added in three portions during 1 hr. at room temperature. A little acetone was also added during the methylation. The solution was then heated on the water bath for 20 min. to remove acetone and destroy excess methyl sulphate, the solution being kept alkaline by small additions of NaOH . During the heating an oil separated and, on keeping the solution at 0° , crystals separated and the oil solidified. The solid (1 g.) was filtered off, dried and then recrystallized several times from hot water. The purified crystals (250 mg.) were identical with authentic veratrolesulphondimethylamide and formed long rectangular leaflets. (Found: C, 49.0; H, 6.3; S, 12.8%. $\text{C}_{10}\text{H}_{15}\text{O}_4\text{NS}$ requires C, 49.0; H, 6.2; S, 13.1%.) They melted at 112° ,¹ not depressed by the authentic substance, but depressed considerably by authentic anisolesulphondimethylamide. Both the above sample and the synthetic sample of veratrolesulphondimethylamide appear to sinter at about 105° unless very carefully dried before taking the melting point.

Synthetic experiments

Veratrole-4-sulphondimethylamide. Veratrole was converted successively into potassium veratrole-4-sulphonate, veratrole-4-sulphonyl chloride and -4-sulphonamide according to Paul [1906, 1]. The sulphonamide (1 g.) was dissolved in 30 ml. 10% NaOH and methylated with 8 ml. methyl sulphate with vigorous stirring. After about 10 min. the solution became warm and a white solid began to separate. Stirring was continued for another 30 min. and then the alkaline

¹ Melting points are uncorrected.

solution was diluted with water. The white solid was filtered off, thoroughly washed with water and recrystallized from a large volume of hot water. The yield of *veratrole-4-sulphondimethylamide* was almost quantitative. It formed long rectangular leaflets, insoluble in alkali and cold water, sparingly soluble in hot water and soluble in alcohol; m.p. 115°. (Found: C, 49.6; H, 6.4; N, 5.3%. $C_{10}H_{15}O_4NS$ requires C, 49.0; H, 6.2; N, 5.7%.)

Anisole-p-sulphondimethylamide. Phenol-*p*-sulphonamide (1 g.) was shaken with 8 ml. methyl sulphate and 20 ml. 15% NaOH, a little methyl alcohol being added to facilitate mixing. The mixture was shaken for 15 min. and then heated on a water bath for about 15 min. During this time an oil separated which solidified on cooling. The solid *anisosulphondimethylamide* was recrystallized from a large volume of hot water. It formed plates, m.p. 75°. (Found: C, 50.4; H, 5.9%. $C_9H_{13}O_3NS$ requires C, 50.2; H, 6.1%.)

1:2-Diacetoxybenzene-4-sulphonyl chloride. 5 g. of well-powdered catechol were added to 6 ml. conc. H_2SO_4 . The catechol quickly dissolved and the viscous solution was kept at 0° for a week. By this time the mixture had become an almost solid crystalline mass. The latter was broken up and dissolved in about 300 ml. water and the excess of H_2SO_4 removed with $BaCO_3$. The solution was boiled and filtered from $BaSO_4$. Any barium left in the filtrate was precipitated by adding the requisite amount of 5*N* H_2SO_4 and filtering. The filtrate, containing the catecholsulphonic acid, was evaporated to a syrup *in vacuo* at 50–60°; the syrup was then dried. No attempt was made to crystallize the acid (Cousin [1893] states that on evaporating a solution in a desiccator, catecholsulphonic acid forms crystals, m.p. 53–54°). The dry syrup was dissolved in 12 ml. pyridine with cooling under the tap and the solution treated with 12 ml. acetic anhydride, again with cooling. The mixture was kept overnight at room temperature. The resulting thick liquid was concentrated *in vacuo* at 50° to a stiff syrup which did not crystallize. This product is, presumably, *pyridine 1:2-diacetoxybenzene-4-sulphonate*; it was easily soluble in water and its solution gave no coloration with $FeCl_3$. The syrupy pyridine salt was now triturated with 12 g. powdered PCl_5 added in small portions. There was a vigorous reaction with much heat. The resulting mobile yellow liquid was poured into 1 l. cold water and stirred until it solidified. The solid was broken up and kept several hours in the water. The water was now changed and, after finely powdering, the whole was kept overnight. The *1:2-diacetoxybenzene-4-sulphonyl chloride* was filtered off and dried (yield, crude, 10.3 g.). It was recrystallized from carbon tetrachloride and formed large elongated hexagonal plates, m.p. 116°. (Found: C, 41.1; H, 2.9; S, 10.65; Cl, 12.1%. $C_{10}H_9O_6SCl$ requires C, 41.0; H, 3.1; S, 10.95; Cl, 12.1%.) On keeping even the pure chloride for about 2–3 months, it decomposed leaving a syrupy fuming mass.

Catechol-4-sulphonamide. 5 g. of the foregoing chloride were stirred into 20 ml. of a solution of equal parts water and ammonia solution (sp. gr. 0.880). The chloride gradually dissolved and the solution was kept overnight. The solution was now acidified with 3*N* HCl, thereby becoming cloudy, and oily globules separated on standing. The solution was extracted with ether in a continuous extractor for 12 hr. Evaporation of the ether left a clear brown resin (2 g.) which could not be crystallized. Its aqueous solution gave, with ferric chloride, an intense green colour turning blue-green, purple and finally red on adding dilute Na_2CO_3 , and it reduced ammoniacal $AgNO_3$ in the cold. The resin was *catechol-4-sulphonamide*, since on methylation with methyl sulphate and alkali it gave *veratrole-4-sulphondimethylamide*, m.p. 113° not depressed by an authentic sample.

1:2-Diacetoxybenzene-4-sulphonanilide. A saturated solution of the above chloride (1 mol.) in purified ethyl acetate was mixed with a saturated solution of aniline (2 mol.) in the same solvent [cf. Fosbinder & Walter, 1939]. The mixture was kept at room temperature overnight. During this time the hydrochloride of the base crystallized. This was filtered off and the filtrate evaporated on the water bath. The residual oil solidified on stirring with water and the solid was recrystallized from aqueous alcohol. The yield was almost quantitative. The *sulphonanilide* formed elongated hexagonal plates, m.p. 127–128°. (Found: S, 9.0%. $C_{16}H_{15}O_6NS$ requires S, 9.2%.) *1:2-Diacetoxybenzene-4-sulphon-m-chloranilide* was prepared in a similar manner from *m*-chloroaniline. It formed needles from aqueous alcohol, m.p. 153°. (Found: S, 8.3; Cl, 9.4%. $C_{16}H_{14}O_6NSCl$ requires S, 8.35; Cl, 9.2%.)

1:2-Diacetoxybenzene-4-sulphon- β -naphthylamide was similarly prepared from β -naphthylamine. It formed long plates from aqueous methyl alcohol, m.p. 131°. (Found: S, 8.05%. $C_{20}H_{17}O_6NS$ requires S, 8.0%.)

Catechol-4-sulphonanilide. Diacetoxybenzenesulphonanilide was dissolved in cold 5% NaOH. After 5–10 min. the solution was acidified with dilute HCl and the *catecholsulphonanilide* separated on standing. The crystals were filtered off, dried and recrystallized from carbon tetrachloride (yield almost quantitative). It formed white glistening plates, m.p. 225° (decomp.), very sparingly soluble in water and had no action on a 1 in 50 blood solution at room temperature during 24 hr. (Found: C, 54.1; H, 4.4; N, 5.3%. $C_{12}H_{11}O_4NS$ requires C, 54.3; H, 4.2; N, 5.3%.)

Catechol-4-sulphon-m-chloranilide was similarly prepared and formed plates from hot water, m.p. 177°. (Found: C, 48.25; H, 3.7; N, 4.7%. $C_{12}H_{10}O_4NSCl$ requires C, 48.1; H, 3.4; N, 4.7%.)

Catechol-4-sulphon- β -naphthylamide was similarly prepared and formed plates from water with a little alcohol, m.p. 218° (decomp.). (Found: C, 61.3; H, 4.2; N, 4.5%. $C_{16}H_{13}O_4NS$ requires C, 60.9; H, 4.1; N, 4.4%.) The above catechol derivatives were all very sparingly soluble in cold water, but the solutions gave a green colour with $FeCl_3$ and reduced ammoniacal silver nitrate in the cold. They did not reduce Fehling's solution.

SUMMARY

It has been shown that when phenol-*p*-sulphonamide is fed to rabbits, a small portion is oxidized to catechol-4-sulphonamide. The latter was isolated from acid-hydrolysed urines and characterized as the crystalline methylated derivative, veratrole-4-sulphondimethylamide, a synthetic sample of which had been made from veratrole-4-sulphonamide, a compound of known structure.

About 1% of the phenolsulphonamide fed was actually accounted for, as pure veratrolesulphondimethylamide, but it is probable that the actual amount oxidized is about 4–5%.

The synthesis of catechol-4-sulphonamide is described. It was found to be a non-crystalline, resinous substance. Crystalline derivatives such as catechol-sulphonanilide etc. have also been described.

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