636. *Some Sulphate Esters of Nitroquinol and 4-Nitrocatechol.*

By J. N. Smith.

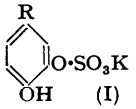
Sulphate esters of nitroquinol (II, III; R = NO_2) and of 4-nitrocatechol (I; R = NO_2) have been prepared by persulphate oxidation of nitrophenols. The absorption spectra of these and of their reduction products have been recorded.

Work in progress in this laboratory on the metabolism of aniline and nitrobenzene required the synthesis of certain sulphate monoesters of dihydroxyanilines. Furthermore, the properties of the sulphate esters of 4-nitrocatechol and nitroquinol which had been prepared in solution in connection with the metabolism of nitrophenols (Robinson, Smith, and Williams, *Biochem. J.*, 1951, **48**, xxvii) seemed to merit further examination as possible enzyme substrates. In the present work, these esters have been isolated as crystalline potassium salts and some of them found to be convenient substrates for the enzyme arylsulphatase (Robinson, Smith, and Williams, unpublished work).

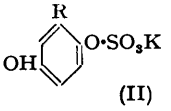
Phenol hydrogen sulphates are well-known compounds and their importance as detoxication products of phenols in the animal body has been recognised for many years (Williams " Detoxication Mechanisms," Chapman and Hall, 1947). They are usually prepared synthetically by the action of sulphur trioxide or chlorosulphonic acid on the phenol in the presence of a tertiary base (*e.g.*, Sobell and Spoerri, *J. Amer. Chem. Soc.*, 1942, **64**, 361; Burkhardt and

Wood, *J.*, 1929, 141). The sulphate esters are also known to be intermediates in the persulphate oxidation of phenols (Elbs, *J. pr. Chem.*, 1893, **48**, 179; Baker and Brown, *J.*, 1948, 2303; Forrest and Petrow, *J.*, 1950, 2340), and the early German patents (D.R.-P. 81,068, 81,297, 81,298; Friedländer, "Fortschritte der Teerfarbenfabrikation," 1894—1897, **4**, 126) claimed the preparation of a number of these compounds by this method though only the preparation of quinol sulphate was described in detail and supported by an analysis. The persulphate oxidation was used in the present work as it offered a convenient means of preparing a monosulphate of a dihydroxyphenol with a free hydroxyl group *para* to the sulphate ester group (cf. Baker and Brown, *loc. cit.*).

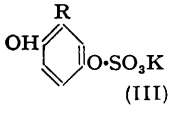
The potassium salts reported here are stable compounds in the dry state and resist hydrolysis by 0·1N-hydrochloric acid at room temperature for 24 hours. In common with other phenol hydrogen sulphates, they are rapidly decomposed by boiling 2N-hydrochloric acid to the corresponding dihydric phenol. The structure of the various compounds follows from this fact and the nature of the starting material (*p*-, *m*-, or *o*-nitrophenol). On reduction with ferrous hydroxide the nitro-compounds yield the corresponding amino-derivatives (I, II; R = NH_2) which slowly become discoloured in air.



(I)



(II)



(III)

Burkhardt and Wood (*J.*, 1929, 141) have observed that dilute nitrous acid readily decomposes *o*-aminophenyl hydrogen sulphate to *o*-aminophenol and sulphuric acid, the solution becoming deep yellow as a result of the action of nitrous acid on the liberated *o*-aminophenol. It has now been found that (II; R = NH_2) which is an *o*-aminohydroxyphenyl hydrogen sulphate behaves similarly with nitrous acid.

The absorption spectra of these sulphates have been determined and compared with those of the relevant nitro- and amino-phenols (see Table). The spectra of the monosulphates (I, II, and III; R = NO_2) differ markedly from those of nitroquinol and 4-nitrocatechol. Nitroquinol in 0·1N-alkali shows a band at 540 mμ. whereas its monosulphates (II and III; R = NO_2) in the same solvent show negligible absorption in this region. 4-Nitrocatechol in alkali shows a peak at 510 mμ. whereas its monosulphate, 2-hydroxy-5-nitrophenyl hydrogen sulphate (I;

	In 0·1N-HCl.					
	$\lambda_{max.}$	$\epsilon \times 10^{-3}$	$\lambda_{max.}$	$\epsilon \times 10^{-3}$	$\lambda_{max.}$	$\epsilon \times 10^{-3}$
p-Nitrophenol	225	6·9	—	—	318	9·8
(I; R = NO_2)	—	—	—	—	315	8·6
4-Nitrocatechol	245	6·3	—	—	345	6·0 ‡
o-Nitrophenol	—	—	280	6·1	352	3·0
(III; R = NO_2)	—	—	273	5·9	360	2·9
2-Nitroquinol	—	—	280	5·5	395	2·8
(II; R = NO_2)	—	—	265	2·6	335	1·8
m-Nitrophenol	225	7·6	275	5·7	320	1·9
p-Aminophenol	—	—	272	1·3	—	—
(I; R = NH_2)	—	—	274	2·0	—	—
m-Aminophenol	—	—	270	1·8	—	—
(II; R = NH_2)	—	—	276	2·0	—	—

	In 0·1N-NaOH.					
	$\lambda_{max.}$	$\epsilon \times 10^{-3}$	$\lambda_{max.}$	$\epsilon \times 10^{-3}$	$\lambda_{max.}$	$\epsilon \times 10^{-3}$
p-Nitrophenol	228	4·5	—	—	402	17·0
(I; R = NO_2)	—	—	—	—	407	18·3
4-Nitrocatechol	—	—	(350	5·8	510	10·3) §
o-Nitrophenol	—	—	282	3·9	412	4·5
(III; R = NO_2)	225	16·8	279	3·9	423	4·8
2-Nitroquinol	238	12·7	—	—	540	3·9
(II; R = NO_2)	235	17·1	—	—	380	1·4
m-Nitrophenol	252	11·2	290	4·2	392	1·58
p-Aminophenol	(234	8·0	303	2·8) *	—	—
(I; R = NH_2)	(230	6·7	298	2·3) †	—	—
m-Aminophenol	—	—	(286	3·2) *	—	—
(II; R = NH_2)	(222	7·4	285	2·7) †	—	—

* In ethanol (Morton and McGookin, *J.*, 1934, 901).
† In water.
‡ In ethanol.
§ In 0·05N-NaOH.

R = NO_2) shows very little absorption in this region, a fact which makes it eminently suitable as a substrate for the assay of arylsulphatase. It is interesting further that the spectra of the isomeric sulphates (II and III; R = NO_2) of nitroquinol also differ from one another. The spectrum of (III; R = NO_2) is very like that of *o*-nitrophenol and it is in fact a sulphated *o*-nitrophenol. Although (II; R = NO_2) is a sulphated *m*-nitrophenol, its spectrum resembles neither that of *m*- nor that of *o*-nitrophenol; a possible explanation is that we have here an instance of steric inhibition of resonance resulting from the presence of the large ·O·SO_3· group *ortho* to the nitro-group, the latter being thus prevented from becoming coplanar with the benzene ring. The spectrum of (I; R = NO_2) is similar to that of *p*-nitrophenol and this substance is in fact a sulphated *p*-nitrophenol. In (I and III; R = NO_2) the nitro- and hydroxyl groups are *para* or *ortho* to each other and the introduction of the ·O·SO_3· groups does not greatly affect the absorption spectrum of the *p*- and *o*-nitrophenols. The spectra of the amino-derivatives (I and II; R = NH_2) correspond to those of *o*- and *m*-aminophenols.

Experimental.

Absorption spectra were measured in a Unicam Spectrophotometer, model SP500. M. p.s are uncorrected.

The nitrophenol (30 g.) was set aside at room temperature for 48 hours in 1 l. of water containing potassium hydroxide (70 g.) and potassium persulphate (70 g.). The dark solution was then acidified to pH 4 with 2N-sulphuric acid, and the free phenol extracted with ether. Neutralisation with potassium carbonate and evaporation of the aqueous solution *in vacuo* at 50—60° left a solid residue which was extracted with cold acetone–water (2 : 1 v/v; 1 l.). The acetone solution was evaporated *in vacuo*, leaving a tarry residue of the sulphate ester which, after crystallisation from a small volume of water, was free from inorganic sulphate and phenol.

Potassium 2-Hydroxy-5-nitrophenyl Sulphate.—The *salt* (I; R = NO_2) (yield, 6 g. from 30 g. of *p*-nitrophenol) crystallised as brown leaflets (decomp. 245—280°), soluble to the extent of about 2% in water at 20° (Found: C, 26·2; H, 1·8; N, 4·3; K, 14·2. $C_6H_4O_7NSK$ requires C, 26·4; H, 1·5; N, 5·1; K, 14·3%). It gave a red colour with ferric chloride. On hydrolysis with 2N-sulphuric acid, followed by ether extraction, it yielded 4-nitrocatechol (m. p. and mixed m. p. 176°) almost quantitatively.

Potassium 4-Hydroxy-3-nitrophenyl Sulphate.—The *salt* (III; R = NO_2) (Found: C, 26·6; H, 1·7; N, 4·7%) (yield, 12 g. from 30 g. of *o*-nitrophenol) crystallised as deep yellow prisms (decomp. 256—257°), showing a solubility of about 2% in water at 20°. A small sample was hydrolysed as above and the liberated phenol examined chromatographically according to the method of Robinson, Smith, and Williams (*Biochem. J.*, in the press) which permits separation of nitroquinol from 3-nitrocatechol. Only nitroquinol was present and in a larger experiment this was isolated in almost quantitative yield (m. p. and mixed m. p. 132°).

Potassium 4-Hydroxy-2-nitrophenyl Sulphate.—The salt (II; R = NO_2) (8 g. from 30 g. of *m*-nitrophenol) crystallised as lemon-yellow prisms of the *monohydrate* which had no sharp decomposition point (Found: C, 24·9; H, 2·4; N, 4·5. $C_6H_4O_7SK,H_2O$ requires C, 24·7; H, 2·1; N, 4·8%). It gave a wine-red colour with ferric chloride and was soluble to about 10% in water at 20°. The phenol liberated on acid hydrolysis was shown by paper chromatography to be free from 3- or 4-nitrocatechol, and 2-nitroquinol was isolated in almost quantitative yield (m. p. and mixed m. p. 132°).

The above compounds were reduced by heating them (1·5 g.) on a water-bath for 40 minutes with crystalline ferrous sulphate (15 g.) and potassium carbonate (9 g.) in water (150 ml.). Filtration and evaporation left residues of the aminophenol sulphates which were extracted with acetone–water (2 : 1 v/v) till all amino-compounds were removed. The extracts were evaporated to dryness and the residues crystallised from a little water or ethanol–water containing a trace of sodium dithionite.

Potassium 5-Amino-2-hydroxyphenyl Sulphate.—The salt (I; R = NH_2) [(0·7 g. from 1·5 g. of (I; R = NO_2)] crystallised as colourless needles of the *monohydrate* (decomp. 205—210°) (Found: C, 27·2; H, 3·2; N, 5·4; K, 15·1. $C_6H_6O_5NSK,H_2O$ requires C, 27·6; H, 3·1; N, 5·4; K, 15·0%), moderately soluble in water and giving a wine-red colour with ferric chloride. It reduced cold neutral silver nitrate, and gave a deep blue colour in the diazo-reaction (nitrous acid, followed by ammonium sulphamate and dimethyl-α-naphthylamine).

Potassium 2-Amino-4-hydroxyphenyl Sulphate.—The *salt* (II; R = NH_2) [(1·5 g. from 2 g. of (II; R = NO_2)] crystallised as colourless prisms which darkened when kept and were moderately soluble in water (Found: C, 29·7; H, 3·0; N, 5·6. $C_6H_6O_5NSK$ requires C, 29·6; H, 2·5; N, 5·8%). It gave a yellow colour on diazotisation, followed by a red colour on coupling with dimethyl-α-naphthylamine, and a deep blue colour with dichloroquinonechloroimide and sodium hydrogen carbonate. It did not reduce cold silver nitrate.

Reduction of (III; R = NO_2) with ferrous hydroxide yielded a product which could not be purified since it rapidly darkened on exposure to air.

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