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RESEARCH ESTABLISHMENT
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THE PREPARATION OF m- AND p- HYDROXY BENZOIC ACIDS (C14 CARBOXYL) BY RADIOLYSIS OF AN AQUEOUS SOLUTION OF BENZOIC ACID (C14 CARBOXYL)

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ABSTRACT

(C14 carboxyl) m- hydroxy-benzoic acid is not available commercially. It was synthesized in low yield by radiolysis of an oxygenated aqueous solution of (C14 carboxyl) benzoic acid, and was separated from unchanged benzoic acid on a cellulose column, and from other radiolytic products by thin-layer chromatography. The para isomer was recovered in the same process.

The radiochemical purity of the products was checked using a paper chromatographic separation.

CONTENTS

1. INTRODUCTION

During a study of radiolytic decarboxylation in these laboratories (Matthews and Sangster 1965), the need arose for some labelled (C14 carboxyl) m- and p-hydroxy-benzoic acids. Because they are not available commercially and synthesis by conventional methods involves several chemical steps for each product, a synthesis was attempted by γ-irradiation of an aqueous, oxygenated solution of (C14 carboxyl) benzoic acid.

Irradiation of a 1 mM solution of benzoic acid produces initially o-, m- and p- hydroxy-benzoic acids as major products. Further attack by .OH radicals on these phenolic products would be expected at positions ortho and para to the hydroxyl groups, producing 2,3-, 2,5-, and 3,4- dihydroxy-benzoic acids. However, Loeff and Swallow (1964) also found 3,5-, 2,4- and 2,6- isomers. They refer to unpublished observations by Capellos and Swallow that salicylic acid yields the 2,3-, 2,4-, and 2,5- dihydroxy benzoic acids, that the m-hydroxy isomer gives 2,3-, 3,4-, and 2,5- di-hydroxy-benzoic acids, and that p- hydroxy-benzoic acid gives 3,4- di-hydroxy benzoic acid. Downes (1958) found only 2,5-, and 2,3-dihydroxy benzoic acids as products from irradiation of aqueous salicylic acid, and states that the 2,4- and 2,6- isomers were not formed.

The G values (molecules formed per 100 eV absorbed) for primary and secondary products are summarized in Table 1. Considerable differences occur in reported results. Taking the mean values and assuming the reaction rates for all aromatics with the hydroxyl radical to be equal, yields may be estimated for the 1.64 mM benzoic acid used in the preparation. These are shown in the last column of Table 1.

This paper describes the irradiation of a solution of benzoic acid and the separation of the m- and p- hydroxy benzoic acid isomers from the parent benzoic acid and the other radiolytic products.

2. METHOD

2.1 Irradiation

Benzoic acid (C14 carboxyl) (Amersham Radiochemical Centre) was diluted with carrier benzoic acid (B.D.H. AR grade) and re-crystallized from ethanol. The product had an activity of 0.5 μCi mg^{-1}. From this labelled material 500 ml of a 1.64 mM solution was prepared using triple distilled water. The solution was adjusted to pH 8 and saturated with oxygen.

Gamma-irradiation was carried out in a spent fuel element assembly. The dose absorbed was 100 krad as determined by the Fricke dosimeter. The rate of formation of mono-hydroxy derivatives is lower after the depletion of oxygen, which occurs at approximately 35 krad. At doses beyond about 100 krad mono-hydroxy acids compete with the benzoic acid for .OH radicals, and form di-hydroxy derivatives.

2.2 Separation

Paper chromatography is impractical on a semi-micro preparative scale. Excessive amounts of unchanged benzoic acid were removed by column chromatography on cellulose. The hydroxy acids were separated by thin layer chromatography on silica gel, which allows treatment of milligram quantities. Paper chromatography was used to check the purity of separated products.

2.3 Column Chromatography

Although inadequate for a complete resolution of the mixture, column chromatography could be used to separate the bulk of unchanged benzoic acid from the phenolic products. Since benzene dissolves relatively more benzoic acid than polar hydroxy compounds, it was used as a constituent of the eluting solvent. Preliminary work showed that cellulose was superior to silica, kieselguhr and alumina as stationary phase, and that a rapid separation could be obtained on a 20 x 1 cm Whatman Standard Grade Cellulose packed column using hexane/benzene (95/5) as eluant.

The irradiated solution was acidified and ether extracted. This concentrated ether extract was transferred to the dry column, excess ether evaporated off, and elution begun with 40 ml of hexane/benzene. Monitoring of successive eluant samples by thin layer chromatography showed the presence only of benzoic acid plus a trace of salicylic acid. (Elution of the latter acid is probably due to the lowered polarity caused by hydrogen bonding between adjacent - COOH and -OH groups.) Ninety per cent of the benzoic acid was removed in this way.

Remaining products were eluted from the column with 30 ml 1 N sodium hydroxide. The eluate was acidified, extracted with ether and evaporated to dryness. The residue was dissolved in alcohol prior to a further separation by thin layer chromatography.

2.4 Thin Layer Chromatography

There have been no previous reports of a solvent system capable of separating m-hydroxy benzoic acid from a mixture of mono-and di-hydroxy benzoic acid isomers in a single step. Experiments showed that ethyl acetate/diethylamine/water (7/8/5) was satisfactory. This system was homogeneous and gave compact zones when using pre-equilibrated plates (Davison - to be published). It did not have the disadvantage of forming the second fronts associated with the more commonly used alcohol/acid/water systems. The Rf values found for ascending development in this system are shown in Table 2.

Using the Desaga apparatus silica gel layers 250 μm thick were prepared on 20 x 20 cm glass plates from a slurry of 30 g of Merck silica gel (G) in 60 ml water. The plates were dried at 110°C and pre-equilibrated overnight in an atmosphere saturated with developing solvent vapour.

The alcoholic solution obtained in the previous stage was streaked along the base of the plate using a template and an adapted Agla Syringe. A test mixture was spotted at each side. Ascending development was carried out in S-tanks using the ethyl acetate/diethylamine/water system. The distance travelled by the solvent front was 15 cm.

After drying, the central portion of the plate was covered and the outer vertical edges sprayed with 1 per cent fluorescein solution. The compounds were located under U.V. light as blue fluorescent, or dark absorption spots against a green fluorescent background. Parallel lines were drawn across the plate and the zones corresponding to m- and p- hydroxy benzoic acids removed. The recovered silica gel fractions were eluted with 1 N sodium hydroxide solution. The eluate was acidified, extracted with ether and the solid hydroxy benzoic acid recovered on evaporation to dryness.

2.5 C14 Determination

Carbon 14 was determined in a Nuclear Enterprises Ltd. single channel liquid scintillation counting assembly. The scintillator solution was 5.3 g of P.P.O. and 0.13 g of P.O.P.O.P. dissolved in 1 litre of ethanol/toluene solvent (volume ratio 44.5/55.5).

2.6 Radiochemical Purity

Possible product contamination could arise from non-fluorescent decomposition products of benzoic acid or phenolic compounds (other than those previously mentioned) which may have been carried right through the separation. This was checked by chromatographing a sample of each isomer on Whatman No. 1 chromatography paper. Since the T.L.C. solvent had been basic an acidic system was chosen.

The organic phase from a benzene/acetic acid/water (2 / 2 / 1) mixture was used as solvent. The paper was equilibrated in the tank with the aqueous phase for 1 hour prior to running the chromatogram. After development and drying the chromatogram strips were cut into 1 cm sections at right angles to the direction of solvent flow. The activity on each section was determined by liquid scintillation counting. Histograms (Figure 1) were constructed and indicate that both products were radiochemically pure.

3. RESULTS AND CONCLUSION

Overall conversion of benzoic acid to m- and p- hydroxy-benzoic acids was 1.75 per cent and 1.10 per cent respectively. These yields correspond to G values of 0.28 and 0.18 as compared with estimates of 0.33 in Table 1. The specific activity of the products would be the same as the starting material (0.5 μCi mg^{-1})

This report shows the feasibility of using radiation-initiated reactions in aqueous solution to form hydroxylated products. Ninety per cent of the unchanged benzoic acid may be recovered. The method therefore is potentially useful for the synthesis of labelled compounds particularly when synthesis by other means involves several complex chemical steps.

4. ACKNOWLEDGEMENTS

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5. REFERENCES

Armstrong, W. A., Black, B. A., and Grant, D. W. (1960). - J. Phys. Chem. 64 : 1415.
Downes, A. M. (1958). - Aust. J. Chem. 11 : 154.
Leoff, I., and Swallow, A. J. (1964). - J. Phys. Chem. 68 : 2470.
Matthews, R. W., and Sangster, D. F. (1965). - J. Phys. Chem. 69 : 1938.

TABLE 1

G VALUES USED TO ESTIMATE PRODUCT YIELDS FROM IRRADIATION OF 1.64 mM AQUEOUS BENZOIC ACID SOLUTION

Products		G Values*			Average G	Estimated Post-Irradiation Concentration for Dose of 100 krad (mM)
		(a) pH 4 1.15 mM ‡	(b) pH 6.5 1.2 mM ‡	(c) pH 6.8-8.3 1.0 mM ‡		
mono-hydroxy benzoate	o-	0.74	0.67	0.37	0.59	0.0610
	m-	0.42	0.37	0.21	0.33	0.0340
	p-	0.33	0.37	0.19	0.30	0.0310
di-hydroxy benzoate	2,3-	-	-	0.031	0.031	0.0032
	2,5-	-	-	0.018	0.018	0.0019
	2,4-	-	-	0.048	0.048	0.0050
residual benzoic acid					G(-BA)~2.4	1.4000

* (a) taken from Downes (1958)
(b) taken from Armstrong et al. (1960)
(c) taken from Leoff and Swallow (1964)

‡ initial concentration of benzoate or benzoic acid

TABLE 2

Rf VALUES FOR HYDROXY-BENZOIC ACIDS IN ETHYL ACETATE/DIETHYLAMINE/WATER (7/8/5) SOLVENT USING PRE-EQUILIBRATED PLATES

	Rf x 100	Fluorescein + U.V.
Benzoic acid	59	Absorbs
o - hydroxy benzoic acid	73	Blue
m - hydroxy benzoic acid	46	Blue
p - hydroxy benzoic acid	28	Absorbs
2,3, - di-hydroxy benzoic acid	56	Absorbs
2,5 - di-hydroxy benzoic acid	61	Green
3,4 - di-hydroxy benzoic acid	16	Absorbs

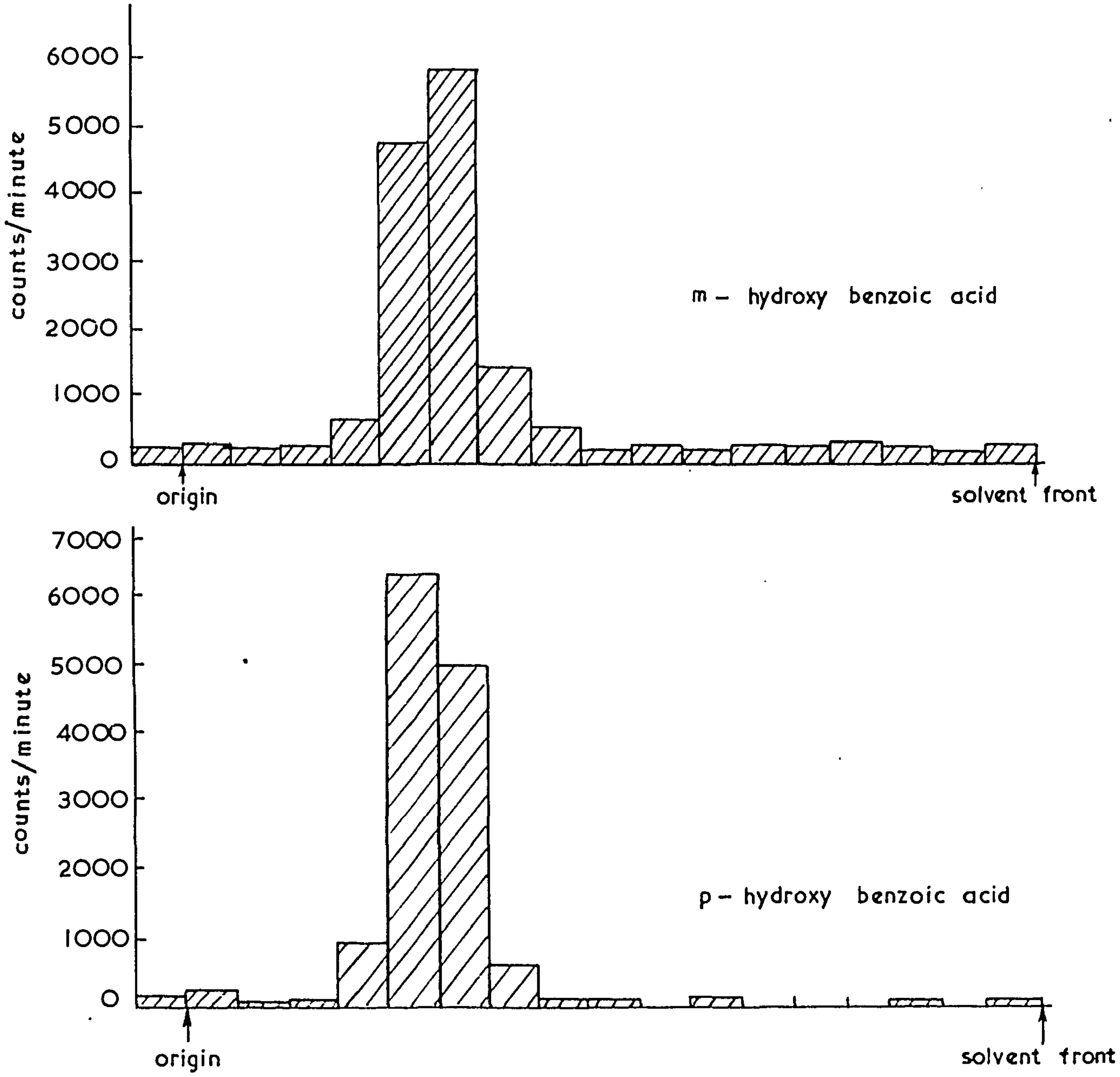

FIGURE 1. HISTOGRAMS SHOWING RADIOCHEMICAL PURITY OF PRODUCTS