

**671.** *The Carboxylation of Resorcinol and the Separation of  $\beta$ - and  $\gamma$ -Resorcylic Acid by Ion-exchange Chromatography.*

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Yields of  $\gamma$ -resorcylic acid afforded by various modifications of the Kolbe-Schmitt reaction have been explored and methods of separating the acid from the accompanying  $\beta$ -isomer have been studied. Selective elution of the acids from a column of an anion-exchange resin in the chloride form with solutions of hydrochloric acid in water or aqueous alcohol effects complete separation. A process for the partial conversion of  $\beta$ - into  $\gamma$ -resorcylic acid is reported.

SALIENT information on the Kolbe-Schmitt reaction (Kolbe, *Annalen*, 1859, **113**, 126; 1860, **115**, 157; Schmitt, *J. pr. Chem.*, 1885, **31**, 397) for the carboxylation of phenols has been summarised by Cameron, Jeskey, and Baine (*J. Org. Chem.*, 1950, **15**, 233), and Wessely, Benedikt, Bengel, Friedrich, and Prillinger (*Monatsh.*, 1950, **81**, 1071). Conflicting claims have been made for the preparation of  $\beta$ - and  $\gamma$ -resorcylic acid by this reaction (Senhofer and Brunner, *Sitzungsber. Akad. Wiss. Wien*, 1879, **80**, II, 504, 514; *Jahresber. Fortschr. Chem.*, 1880, 835; Bistrzycki and von Kostanecki, *Ber.*, 1885, **18**, 1985; Brunner, *Annalen*, 1907, **351**, 320; *Monatsh.*, 1928, **50**, 216; Mauthner, *J. pr. Chem.*, 1930, **124**, 322; von Hemmelmayr, *Monatsh.*, 1917, **38**, 81; Clibbens and Nierenstein, *Org. Synth.*, **10**, 94). The nature of the alkali salt is known to influence both the course and extent of the reaction (Ferguson, Holmes, and Calvin, *J. Amer. Chem. Soc.*, 1950, **72**, 5315; Cameron *et al.*; Wessely *et al.*, *loc. cit.*; Morgan, *J. Soc. Chem. Ind.*, 1931, **50**, 104) but it is not clear from earlier work whether this is so for resorcinol.

Cartwright, Jones, and Marmion (preceding paper) studied various preparative methods for  $\gamma$ -resorcylic acid and investigation of the direct carboxylation of resorcinol is reported in the present paper.

Heating resorcinol with excess of potassium or sodium hydrogen carbonate in water for a short period afforded exclusively  $\beta$ -resorcylic acid, the potassium salt giving the higher yield. Passage of a rapid stream of carbon dioxide through the mixture did not greatly influence the result. Prolonging the treatment with potassium hydrogen carbonate and carbon dioxide gave some  $\gamma$ -resorcylic acid: *e.g.*, after 41 hours 50% and 36% of  $\beta$ - and  $\gamma$ -acid respectively were obtained. Use of a higher temperature, by substitution of glycerol for water as the reaction medium, with potassium hydrogen carbonate did not materially influence either the course or extent of the reaction. On the other hand, increasing the pressure of carbon dioxide to 27 atms. speeded the reaction and afforded quantitative carboxylation at 120–130°, 48% of the product being  $\gamma$ -resorcylic acid. Much the same results were obtained whether the mono- or the di-potassium derivative of resorcinol was used. The lithium derivatives gave less carboxylation, both  $\beta$ - and  $\gamma$ -acid being formed; ammonium hydrogen carbonate yielded exclusively  $\beta$ -acid. Carboxylation of the anhydrous disodium or dipotassium salt of resorcinol in benzene gave virtually quantitative yields of acid, but only with the dipotassium salt could  $\gamma$ -resorcylic acid be detected, and then only a trace. Carboxylation of resorcinol at 175° with potassium carbonate and carbon dioxide (60 atms.) according to Marassé's technique (G.P. 76,441/1894) led to 90% conversion,  $\gamma$ -acid comprising about 15% of the product. Sodium carbonate, in like manner, furnished only 35% of acids, only 4% of which was the  $\gamma$ -isomer. An attempt to carboxylate resorcinol in pyridine at 160–170° with carbon dioxide (50 atms.) was unsuccessful.

It appears from some of the experiments that the first product is the  $\beta$ -acid,  $\gamma$ -resorcylic acid being subsequently formed by rearrangement. *E.g.*, when the mono-sodium or -potassium salt of  $\beta$ -resorcylic acid was heated in water at 200° part of the acid (26.6% and 19.5% respectively) was recovered as the  $\gamma$ -isomer. Resorcinol was also formed and carbon dioxide liberated. This rearrangement of  $\beta$ - to  $\gamma$ -resorcylic acid through its alkali metal salt is analogous to the formation of dipotassium *p*-hydroxybenzoate, phenol, and carbon dioxide from potassium salicylate (Ost, *J. pr. Chem.*, 1875, [ii], **11**, 24, 385). Unlike sodium salicylate, sodium  $\gamma$ -resorcylicate also undergoes rearrangement.

$\beta$ - and  $\gamma$ -Resorcylic acid may be separated, although somewhat tediously, by fractional crystallisation at  $\gt 80^\circ$ . Above  $80^\circ$   $\gamma$ -resorcylic acid tends to be decarboxylated. Baker (*Nature*, 1936, **137**, 236) has drawn attention to the high acid strength of  $\gamma$ -resorcylic acid compared with the  $\beta$ -acid ( $K\ 5.0 \times 10^{-2}$  and  $0.52 \times 10^{-3}$  respectively; cf. Abichandani and Jatkar, *J. Indian Inst. Sci.*, 1941, **23**, A, 77), which he attributes to the double chelation of the  $\gamma$ -acid anion. It was therefore considered that ion-exchange chromatography should provide convenient separation.

Ion-exchange chromatography has been used extensively for separation of complex mixtures of amphoteric electrolytes such as amino-acids (Partridge, *Chem. and Ind.*, 1950, 383; Moore and Stein, *J. Biol. Chem.*, 1951, **192**, 663) and mononucleotides (Cohn, *J. Amre. Chem. Soc.*, 1950, **72**, 1471), but its use for relatively simple carboxylic acids has received little attention. For the separation of weak electrolytes which differ appreciably in their dissociation constants it is usually convenient to base the separation on differences in the solution equilibria rather than on differences in the affinities of the ions for the ion-exchange resin. Thus Davies (*Biochem. J.*, 1949, **45**, 38) has shown that, for the separation of two weak acids by elution, the best separation will normally be achieved when the pH of the solution applied to the resin is 1–2 units lower than the value of  $\frac{1}{2}(pK + pK')$  where  $K$  and  $K'$  are the dissociation constants of the two acids. The weaker acid will then be less readily taken up by the resin and will be preferentially eluted.

When aqueous eluting agents were employed for the separation of  $\beta$ - and  $\gamma$ -resorcylic acid, large volumes of eluants were required owing to the "tailing" of the bands. This effect which has been frequently observed in the chromatographic separation of organic acids was however overcome by the addition of alcohol to the eluting agent (cf. Moore and Stein, *loc. cit.*, who added butyl alcohol to the eluting agent employed for the removal of the aromatic amino-acids). Separation of the resorcylic acids was most readily achieved by absorbing the mixture on an anion-exchange resin in the chloride form and then selectively eluting the  $\beta$ - and the  $\gamma$ -acid with 0.01N- and 0.1N-hydrochloric acid respectively in 75% ethyl alcohol.

Substitution in 7-hydroxy-4-methylcoumarin, derived from resorcinol by condensation with acetoacetic ester, generally yields 8-substituted derivatives as the initial and principal products. It seemed of interest to see whether the 8-carboxylic acid could be obtained exclusively by carboxylation and whether this would yield  $\gamma$ -resorcylic acid on hydrolysis with strong alkali. Carboxylation of the potassium salt of the coumarin at  $180^\circ$  yielded 47% of 7-hydroxy-4-methylcoumarin-8- plus -6-carboxylic acid. A small amount of resorcinol-2:4-dicarboxylic acid was also formed in one experiment, presumably by hydrolysis followed by carboxylation. The 6-acid has been prepared by Shah, Sethna, Banerjee, and Chakravarti (*J. Indian Chem. Soc.*, 1937, **14**, 717) by condensing  $\beta$ -resorcylic acid with acetoacetic ester in presence of sulphuric acid. An authentic sample of the 8-acid was prepared by us in the same way from  $\gamma$ -resorcylic acid. On hydrolysis with 20% sodium hydroxide solution it afforded some  $\gamma$ -resorcylic acid but considerable decarboxylation occurred simultaneously, yielding resorcinol.

#### EXPERIMENTAL

Microanalyses are by Miss M. Corner.

*Carboxylation of Resorcinol.*—Resorcinol (40 g.) and sodium hydrogen carbonate (200 g.) in water (400 c.c.) were heated on a steam-bath for  $1\frac{1}{2}$  hours and finally boiled. After cooling, the mixture was acidified with hydrochloric acid and extracted with ether. The resorcylic acid was removed from the ethereal extract by aqueous sodium hydrogen carbonate and recovered by acidification and extraction with ether. The crude solid remaining after removal of the solvent, crystallised from water ( $\gt 80^\circ$ ), gave pure  $\beta$ -resorcylic acid (23.5 g.), m. p.  $213^\circ$  (decomp.). With alcoholic ferric chloride solution the acid gave a red colour. No trace of  $\gamma$ -resorcylic acid could be detected. Resorcinol (19.5 g.) was recovered from the original ethereal extract.

Resorcinol (236 g.) and potassium hydrogen carbonate (1500 g.) in water (2 l.), when heated under the same conditions, afforded 302 g. of  $\beta$ -resorcylic acid, and 15 g. of resorcinol were recovered unchanged. Again, no  $\gamma$ -resorcylic acid was detected.

When the directions of Clibbens and Nierenstein (*loc. cit.*) were followed and the mixture was

heated on the steam-bath for 4 hours and then under reflux for  $\frac{1}{2}$  hour, with a rapid stream of carbon dioxide passing through the solution, potassium hydrogen carbonate gave a 57% yield of pure  $\beta$ -acid, 30% of resorcinol being recovered. Similarly, sodium hydrogen carbonate gave a 50% yield of  $\beta$ -acid with 27% recovery of resorcinol. In both experiments substitution occurred exclusively in the  $\beta$ -position.

Resorcinol (75 g.) and potassium hydrogen carbonate (110 g.), dissolved in the minimum amount of water at room temperature, were heated under reflux ( $\sim 110^\circ$ ) for 16 hours with a rapid stream of carbon dioxide passing through the solution. After cooling, the solution was acidified with hydrochloric acid. The precipitated solids were filtered off and washed with cold water, and on crystallisation from hot water gave pure  $\beta$ -resorcylic acid (15.8 g.). The filtrate was exhaustively extracted with ether and the extract treated with sodium hydrogen carbonate solution, which on acidification and further ether-extraction afforded a further quantity of  $\beta$ -acid (8 g. after crystallisation). The filtrate was extracted with ether, the ether removed, and the solid crystallised from boiling benzene and recrystallised from aqueous sodium chloride at  $> 80^\circ$ . In this way, pure  $\gamma$ -resorcylic acid (18.5 g.), m. p.  $163\text{--}165^\circ$  (decomp.), was obtained. With alcoholic ferric chloride solution  $\gamma$ -resorcylic acid gives an intense blue colour. From the original ethereal extract resorcinol (30 g.) was recovered. This method of separating  $\beta$ - and  $\gamma$ -resorcylic acid was generally followed in subsequent experiments.

Acids were obtained in 86% yield by heating resorcinol (110 g.), potassium hydrogen carbonate (500 g.), and water (700 g.) under reflux ( $\sim 110^\circ$ ) for 41 hours with passage of a stream of carbon dioxide, giving  $\beta$ - (77.5 g.) and  $\gamma$ -resorcylic acid (56 g.).

Resorcinol (110 g.) and potassium hydrogen carbonate (200 g.) in glycerol (300 g.) at  $130\text{--}140^\circ$  (22 hours) with admission of a stream of carbon dioxide gave  $\beta$ - (47 g.) and  $\gamma$ -resorcylic acid (23.7 g.).

The following results illustrate the effect, principally, of pressure :

Reactants			Conditions			Products		
Resorcinol (g.)	Alkali (g.)	Water (g.)	Temp.	Max. CO <sub>2</sub> press. (atms.)	Time (hr.)	Resorcinol (g.)	$\beta$ -Acid (g.)	$\gamma$ -Acid (g.)
55	200 KHCO <sub>3</sub>	300	130°	27	3.5	—	39	36
110	400 "	500	93	34	4	35	55.5	44.5
110	140 "	100	120	17	5	—	142	10 *
110	100 "	100	119	18	6.25	5	80	60
156	160 KOH	400	109	13.5	4	52	103	20
110	56 "	200	108	13.5	3	20	66	26

\* The reactants were not brought into solution by warming before insertion in the autoclave, as with the other mixtures. This may account for the seemingly anomalous result.

Lithium carbonate (18.5 g.) and resorcinol (27.5 g.) in water (300 c.c.) were heated under reflux for 8 hours with passage of carbon dioxide. The bulk of the resorcinol (24 g.) was recovered unchanged and the acid product (3 g.) consisted entirely of  $\beta$ -acid. No acid was formed by treatment of resorcinol (23 g.) with lithium carbonate (15 g.) in glycerol (200 c.c.) at  $160\text{--}180^\circ$  for 24 hours (carbon dioxide stream). Failure to effect any appreciable reaction with lithium carbonate may be due to the low solubility of the carbonate. Resorcinol (132 g.) in water (200 c.c.) containing lithium hydroxide (60 g.) at  $110^\circ$  (6 hours) with carbon dioxide (40 atms.) furnished 45 g. of acid, 88 g. of resorcinol being recovered. Fractional crystallisation yielded 33 g. of  $\beta$ - and 8 g. of  $\gamma$ -resorcylic acid. Ammonium hydrogen carbonate (150 g.) and resorcinol (110 g.) in water (400 c.c.) (carbon dioxide, 50 atms.) at  $110\text{--}125^\circ$  (6 hours) furnished only  $\beta$ -resorcylic acid (83 g.).

The disodium salt of resorcinol (21.5 g.) was suspended in benzene and the last traces of water were removed by azeotropic distillation. This suspension in benzene ( $\sim 200$  c.c.) was transferred to an autoclave and heated with carbon dioxide (40 atms.) at  $110\text{--}130^\circ$  for 3 hours, and then for 3 hours at  $130\text{--}140^\circ$ . A nearly quantitative recovery of  $\beta$ -resorcylic acid (30 g.) was obtained. Resorcinol (43 g.), as its dipotassium salt, when similarly treated also gave a theoretical yield of carboxylic acid (60 g.) but in this case a small amount (1 g.) of  $\gamma$ -resorcylic acid was present.

An attempt to carboxylate resorcinol (110 g.) in pyridine (300 g.) solution by heating it with carbon dioxide (40 atms.) at  $160\text{--}170^\circ$  for 6 hours proved unsuccessful, resorcinol (109.4 g.) being recovered unchanged.

In accordance with Marassé's technique (*loc. cit.*) resorcinol (55 g.) was heated with anhydrous potassium carbonate (250 g.) and carbon dioxide (25 atms.). The temperature was raised

slowly from 120° to 175° during 3 hours, the pressure attaining 60 atms. at the final temperature. This results in 89.5% of carboxylation, the product comprising  $\beta$ - (53 g.) and  $\gamma$ -resorcylic acid (10.5 g.). A slightly higher temperature (4½ hours at 150—190°, maximum pressure of carbon dioxide 65 atms.) furnished a 91.5% yield of acid product—56 g. of  $\beta$ - and 9 g. of  $\gamma$ -acid. Anhydrous sodium carbonate (300 g.) and resorcinol (55 g.) when similarly treated (5 hours at 150—184°, 43 atms. carbon dioxide) gave only 35% of acids ( $\beta$ , 24 g.; and  $\gamma$ , 1 g.).

*Rearrangement of  $\beta$ - to  $\gamma$ -Resorcylic Acid.*—When an aqueous solution of sodium  $\beta$ -resorcylic acid (from 77 g. of  $\beta$ -acid and 42 g. of sodium hydrogen carbonate) was heated at 200° for 5 hours the product consisted of a mixture of  $\beta$ - (21 g.) and  $\gamma$ -resorcylic acid (20.5 g.) and resorcinol (13 g.). Carbon dioxide was also generated during the reaction; a pressure of 3 atms. was registered after cooling to room temperature. Similarly, the potassium salt (from 77 g. of  $\beta$ -acid and 50 g. of potassium hydrogen carbonate), dissolved in the minimum quantity of water, when heated for the same period at the same temperature yielded on acidification  $\beta$ -acid (19.5 g.),  $\gamma$ -acid (15 g.), and resorcinol (16.5 g.). Free carbon dioxide liberated during the reaction led to a residual pressure of 4 atms. Repetition of the latter experiment at 166° led to a partial conversion of  $\beta$ -resorcylic acid (38 g.) into  $\gamma$ -acid (11 g.), resorcinol (14.2 g.), and carbon dioxide; part of the starting material (6.5 g.) was recovered unchanged. The presence of carbon dioxide and potassium hydrogen carbonate did not inhibit the reaction. Thus, when a solution of potassium  $\beta$ -resorcylic acid and potassium hydrogen carbonate ( $\beta$ -acid 80 g., potassium hydrogen carbonate 100 g., water 400 g.) was heated at 160° for 5 hours with an initial pressure of carbon dioxide of 7 atms. the product on acidification consisted of unchanged  $\beta$ -acid (34 g.),  $\gamma$ -acid (26 g.), and resorcinol (15 g.). Experiments in aqueous glycerol at 130° indicated that the reaction also takes place to some extent at this temperature.

*Carboxylation of 7-Hydroxy-4-methylcoumarin.*—Potassium hydrogen carbonate (150 g.), 7-hydroxy-4-methylcoumarin (76 g.), and water (300 g.) were heated for 5 hours at 110° with carbon dioxide (40 atms.). The coumarin was recovered unchanged. The same reactants, when heated for 3 hours at 150° with carbon dioxide (50 atms.), gave mixed acids (28.8 g.). After 3 hours at 180° with carbon dioxide (34 atms.) 7-hydroxy-4-methylcoumarin (50 g.) potassium hydrogen carbonate (150 g.), and water (300 g.) furnished a 47% yield of acidic material. The reaction products were treated as follows. After acidification and exhaustive ether-extraction the acids were removed with bicarbonate solution. Acidification of the bicarbonate extract yielded a solid which was filtered off and washed with water. Ether-extraction of the filtrate afforded a little more acid. Crystallisation of the crude acid from aqueous sodium chloride yielded an insoluble residue and colourless crystals, m. p. 201—202° (decomp.) after crystallisation from aqueous alcohol. This was 7-hydroxy-4-methylcoumarin-8-carboxylic acid (Found: C, 60.2; H, 3.7.  $C_{11}H_8O_5$  requires C, 60.0; H, 3.6%). Extraction with ethyl acetate provided better separation, the 8-carboxylic acid being readily soluble therein. The insoluble residue, m. p. 278—282° (decomp.) on crystallisation from aqueous alcohol, gave 7-hydroxy-4-methylcoumarin-6-carboxylic acid, m. p. 282—284° (decomp.) [lit., 284—285° (decomp.)] (Found: C, 60.3; H, 3.8%). An authentic sample, m. p. 282—284° undepressed on admixture with the above, was prepared by the method of Shah *et al.* (*loc. cit.*). The 8-carboxylic acid (5.6 g.), m. p. 201—202° (decomp.), was obtained by similar condensation of  $\gamma$ -resorcylic acid (7 g.) with acetoacetic ester (6 g.) and sulphuric acid. There was no depression of the m. p. when this was mixed with the 8-acid obtained in the carboxylation reaction. This acid gives a blue colour with alcoholic ferric chloride; the 6-carboxylic acid gives a red colour. Some decarboxylation of the  $\gamma$ -resorcylic acid or of 7-hydroxy-4-methylcoumarin-8-carboxylic acid occurred during the condensation since 7-hydroxy-4-methylcoumarin (2 g.) was also isolated.

The crude 6-acid obtained in a carboxylation reaction carried out at 150° was contaminated with a small amount of resorcinol-2 : 4-dicarboxylic acid, m. p. 312° (Found: C, 48.5; H, 3.2. Calc. for  $C_8H_6O_6$ : C, 48.5; H, 3.0%), separated by taking advantage of its low solubility in acetone. This experiment gave the 8- (16.1 g.) and 6-carboxylic acid (10.5 g.) and resorcinol-2 : 4-dicarboxylic acid (1 g.). In a carboxylation at 180° the acid product (29.3 g.) consisted entirely of 8- and 6-acid (22.2 and 7.1 g. respectively).

When the 8-carboxylic acid (5 g.) was heated under reflux with 20% sodium hydroxide solution (20 c.c.) for 2 hours, the product consisted of  $\gamma$ -resorcylic acid (1 g.), resorcinol (1.2 g.), and 7-hydroxy-4-methylcoumarin (0.5 g.).

*Separation of  $\beta$ - and  $\gamma$ -Resorcylic Acid by Ion-exchange Chromatography.*—Resin columns. For preliminary experiments on a small scale, columns of the type described by Djurfeldt and Samuelson (*Acta Chem. Scand.*, 1950, 4, 165) were employed. The bed of resin, 0.5 ml. in

volume ( $4.0 \times 0.4$  cm.), was supported between two glass-wool plugs. For separations on the gram-scale, a larger column with a bed-volume of approx. 20 ml. ( $12 \times 1.4$  cm.) was used. A polyethylene float was used in the column to prevent disturbance of the upper layers of the resin. During a separation the effluent from the column was collected in 10-ml. fractions, by a Shandon automatic fraction collector.

For the greater part of this work the weakly basic resin Amberlite XE-76 (supplied by the Rohm and Haas Co.) was employed. The behaviour of three other anion-exchange resins, Amberlite IRA-400 (Rohm and Haas Co.), De-Acidite B and De-Acidite E (Permutit Co. Ltd.) was also examined. In each case the particle diameter of the resin was 150–250  $\mu$ . Before use the resins were treated with *N*-hydrochloric acid and *N*-sodium hydroxide to remove any soluble matter. They were then converted into the chloride form with *N*-hydrochloric acid and washed to remove excess of regenerant.

*Analysis of column effluent.* Paper chromatography provided a convenient method of qualitative analysis. The procedure adopted was similar to that used by Bray, Thorpe, and

FIG. 1. Apparatus for qualitative analysis of column effluent.

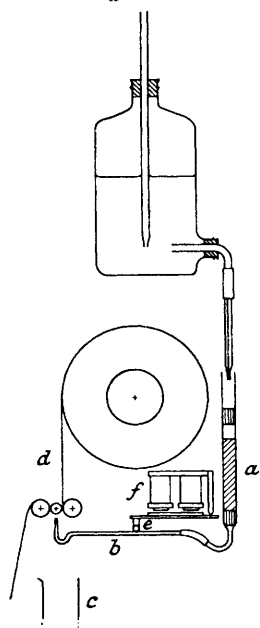
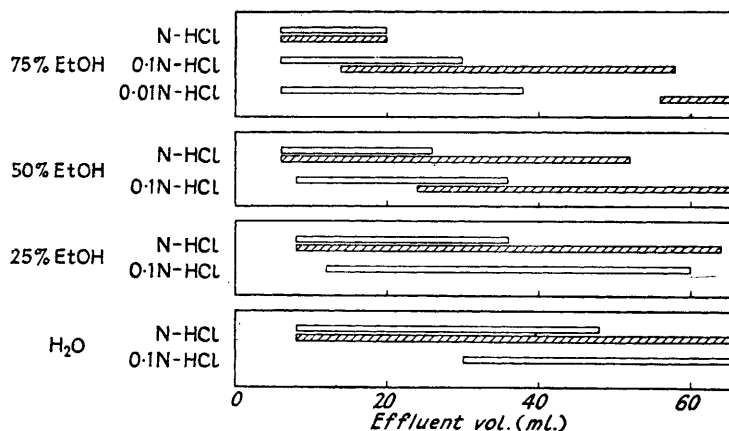


FIG. 2. Effect of eluant. Open rectangles,  $\beta$ -resorcylic acid; hatched rectangles,  $\gamma$ -resorcylic acid.



White (*Biochem. J.*, 1950, **46**, 271). The chromatograms were developed with butanol-acetic acid and after drying were sprayed with a 0.2% solution of ferric chloride in 95% ethyl alcohol.

For the examination of the effluent from the small columns a procedure similar to that described by Drake (*Nature*, 1947, **160**, 602) was used. The apparatus is shown in Fig. 1. The effluent from the column (a) passed through a glass cannula (b) approx. 15 cm. in length and was collected in a receiver (c). A strip of Whatman No. 1 paper (d), 7 cm. wide, was moved past the tip of the cannula by rubber rollers at approx. 0.5 cm. per minute. The tip of the cannula was normally prevented from touching the surface of the paper by a metal bar (e). At intervals of 4 minutes the metal bar was raised momentarily by an electromagnet (f) operated through a time-switch. This allowed a drop of the column effluent from the glass cannula to be collected on the paper strip, about 1 cm. from the edge. At the end of an experiment, the paper was inserted in the spool of a photographic developing tank which was allowed to stand in a Petri dish containing butanol-acetic acid. After approx. 20 minutes, the paper strip was removed from the spool, dried, and sprayed with ferric chloride solution.

For qualitative analysis of the 10-ml. fractions from the larger column, 0.02-ml. samples from the different fractions were placed at 3-cm. intervals on sheets of Whatman No. 1 paper, and the chromatogram developed with butanol-acetic acid, Williams and Kirby's ascending method (*Science*, 1948, **107**, 481) being used.

For quantitative analysis of fractions from the large column, ultra-violet absorption was measured at 2940 and 3060 Å with a Beckman Model DU Spectrophotometer. Aliquot samples from the different fractions were diluted with water and ethyl alcohol to a suitable final concentration of the resorcylic acid and an ethyl alcohol concentration of 3.0%. The extent of cross-contamination in the intermediate fractions could be estimated from the ratio of the optical densities at 2940 and 3060 Å. A constant value for this ratio in samples from consecutive fractions indicated the presence of a single component.

*Aqueous eluants.* Preliminary experiments showed that both resorcylic acids were readily absorbed by Amberlite XE-76 in the chloride form and that they could be eluted with dilute acid or alkali. In all cases the  $\beta$ -acid appeared to be eluted preferentially. Alkaline eluting agents were however unsuitable owing to the instability of the resorcylic acids therein. The acids were therefore eluted with hydrochloric acid solutions. In a typical experiment, a solution containing 2.65 g. of the mixture of resorcylic acids in 500 ml. of water was passed through the 20-ml. column of Amberlite XE-76 in the chloride form at 5 ml. per minute. The  $\beta$ -resorcylic acid was then eluted with 1300 ml. of 0.1N-hydrochloric acid at the same flow rate, the effluent being collected in 10-ml. fractions. When the concentration of hydrochloric acid was increased to 0.5N,  $\gamma$ -resorcylic acid was eluted. A total volume of 1000 ml. of 0.5N-hydrochloric acid was passed through the column, but the  $\gamma$ -resorcylic acid was not completely removed. Fractions

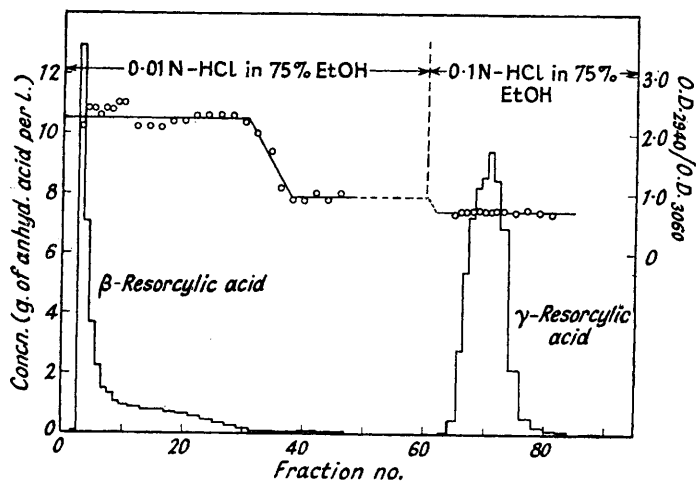


FIG. 3. Separation of  $\beta$ - and  $\gamma$ -resorcylic acid.

1—100 were combined and extracted with ether. On evaporation of the extract, pure  $\beta$ -resorcylic acid was obtained (0.88 g.).  $\gamma$ -Resorcylic acid (1.10 g.) was obtained by ether-extraction of fractions 135—235.

*Non-aqueous eluants.* Although a good separation of the two resorcylic acids was achieved by the method described above, large volumes of eluant were required to remove the acids from the column. It was considered that non-aqueous eluting agents would be more satisfactory since the solubility of the acids would be increased and they could be eluted at a higher concentration. In addition the presence of the organic solvent might result in more favourable distribution coefficients. Preliminary experiments were carried out with hydrochloric acid in mixtures of water with methyl alcohol, ethyl alcohol, *n*-butyl alcohol, acetone, or dioxan. It was confirmed that the presence of the organic solvent markedly reduced the volume of eluant required.

Use of solutions of hydrochloric acid in different mixtures of ethyl alcohol and water as eluting agents was examined in detail. The apparatus for small-scale experiments described above was employed. The column contained a 2-ml. bed of Amberlite XE-76 in the chloride form. A solution of 0.1 g. of the resorcylic acid mixture in 50 ml. of water was passed through the column which was then washed with 10 ml. of de-ionised water. The eluant was passed through the column at 0.5 ml. per minute. The results obtained are shown diagrammatically in Fig. 2. In general, for a given acid concentration, the volume of solution required to elute the resorcylic acids decreased as the proportion of alcohol in the solvent increased. Similarly, for a given alcohol concentration, the volume of eluant required decreased as the concentration of acid increased. It appears from Fig. 2 that 0.01N-hydrochloric acid in 75% (v/v) ethyl alcohol

would be suitable for the elution of the  $\beta$ -resorcylic acid and 0.1N- or N-hydrochloric acid in 75% (v/v) ethyl alcohol for the elution of the  $\gamma$ -resorcylic acid.

The use of these eluting agents for the separation of gram quantities was examined, with the 20-ml. column of Amberlite XE-76. A solution of 1.50 g. of the resorcylic acid mixture in 500 ml. of water was passed through the column at approx. 1.0 ml. per minute. The  $\beta$ -acid was then eluted with 0.01N-hydrochloric acid in 75% ethyl alcohol at 1.5 ml. per minute (total volume, 600 ml.).  $\gamma$ -Resorcylic acid was then eluted with 0.1N-hydrochloric acid in 75% alcohol at the same flow rate. The concentrations of the two resorcylic acids in the different fractions of the effluent are shown in Fig. 3 which also shows the ratio of optical densities (O.D.) of the different fractions at 2940 and 3060 Å. It will be seen from Fig. 3 that the  $\beta$ -resorcylic acid is contained in the first 350 ml. of effluent. The different value for the optical density ratio in the next 250 ml. of effluent indicates the presence of a third component. The presence of this additional component, probably resorcinol-2:4-dicarboxylic acid, was confirmed by paper chromatography; the  $R_F$  value was different from that of the  $\beta$ - and  $\gamma$ -resorcylic acids and a reddish-pink colour was obtained with ferric chloride solution. The  $\gamma$ -resorcylic acid was removed from the column in approx. 200 ml. of the 0.1N-hydrochloric acid. The resorcylic acids and the third component were isolated from the effluent by bulking the appropriate fractions, removing most of the alcohol by distillation *in vacuo*, and extracting the remaining solution with ether. The yields of  $\beta$ - and  $\gamma$ -resorcylic acid were 0.57 and 0.75 g. respectively, and approx. 0.02 g. of the third component was isolated. The m. p. of samples of the two acids which had been dried at  $10^{-4}$  mm. over phosphoric oxide were  $\beta$ - 224° (corr.),  $\gamma$ - 171° (corr.) (both, rapid heating). The titration curve of  $\beta$ -resorcylic acid showed inflexions at pH 6 and about 10.  $\gamma$ -Resorcylic acid showed a single inflexion. The ultra-violet absorption spectra of the two acids, determined with a Beckman Model DU Spectrophotometer, for aqueous solutions containing approx. 0.02 g. of acid per l., showed max. at:  $\beta$ -, 2490 ( $\log \epsilon$  4.00) and 2920 ( $\log \epsilon$  3.66);  $\gamma$ -, 2460 ( $\log \epsilon$  3.79) and 3060 Å ( $\log \epsilon$  3.49), in agreement with Cartwright, Jones, and Marmion (*loc. cit.*).

*Behaviour of different ion-exchange resins.* Small-scale experiments with Amberlite IRA-400, De-Acidite B and De-Acidite E in the chloride form and approx. 0.1 g. of mixed resorcylic acids indicated that equally satisfactory separations could be achieved with them.

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