233. *The Analysis of Mixtures of Benzene-carboxylic Acids by Partition Chromatography.*

By Brian Fishwick.

A method has been devised for the separation, by partition chromatography, of mixtures containing benzene-carboxylic acids, which allows identification and quantitative estimation of the component acids. A column of silica gel, with 0·5N-sulphuric acid as stationary phase, is used. Values of peak effluent volumes are reported for all benzene-carboxylic acids, except terephthalic acid. Methods for the preparation of all the less common benzene-carboxylic acids are described briefly.

During investigation into the chemical constitution of different coal macerals, it became necessary to identify and estimate individual benzene-carboxylic acids from the complex mixture obtained by oxidation of coals.[1] Partition chromatography seemed to offer the best solution.

The separation, by partition chromatography, of mixtures of carboxylic acids has often been reported, but there is little literature on the separation of benzene-carboxylic acids by this method, although Turrizianni and Giovanni [2] have described such a separation of benzoic, phthalic, *iso*phthalic, and trimesic acid. The method now devised for the separation of benzene-carboxylic acids is similar to that described by Marvel and Rands [3] for other non-volatile acids.

Although Marvel and Rands [3] and others [4] have used water as stationary phase, some authors [2, 5] buffered the column with mineral acid to obtain sufficiently sharp peaks. For

[1] Roy and Howard, *J. Amer. Chem. Soc.*, 1952, **74**, 3239.

[2] Turrizianni and Giovanni, *Ricerca sci.*, 1954, **24**, 2099.

[3] Marvel and Rands, *J. Amer. Chem. Soc.*, 1950, **72**, 2642.

[4] Baldwin, Loeblick, and Lawrence, *Analyt. Chem.*, 1954, **26**, 760; Roberts and Martin, *ibid.*, p. 815.

[5] Isherwood, *Biochem. J.*, 1946, **40**, 688; Donaldson, Tulane, and Marshall, *Analyt. Chem.*, 1952, **24**, 185; Bulen, Varner, and Burrell, *ibid.*, p. 187.

the separation of benzene-carboxylic acids, mineral acid (preferably 0·5N-sulphuric acid) is required as stationary phase when *n*-butanol–chloroform is used as mobile phase. The mixture to be separated is best added to the column in methanol–chloroform, as some of the mixture is carried through with the solvent front when it is added in *n*-butanol–chloroform.

Peaks are sharp and well separated, except that only partial separation of phthalic from *iso*phthalic acid takes place although peaks are sufficiently well separated for identification of both acids.

Peak effluent volumes are fairly constant and can be used for identification of the acids.

Recoveries of individual acids after separation (measured by the areas under the corresponding peaks) are close to 100% in most cases, with the exceptions of hemimellitic acid (95%), benzene-1 : 2 : 3 : 5-tetracarboxylic acid (95%), benzenepentacarboxylic acid (89%), and mellitic acid (91%). In these cases losses are reproducible so that satisfactory corrections can be applied.

Losses of acids during partition chromatography, owing to esterification, can be avoided by substituting *tert.*-butyl alcohol [6] or a ketone [7] for *n*-butanol in the eluting solvent. If losses of some benzene-carboxylic acids during chromatography are due to esterification, these losses might be avoided by using either of the above solvent mixtures, although separations might be less sharp with the ketone.

All the less common benzene-carboxylic acids used were prepared in the laboratory. In general these preparations involved oxidation of aromatic hydrocarbons, or suitable ketonic derivatives prepared from aromatic hydrocarbons by Friedel–Crafts reactions. Contrary to reports,[8] the oxidation of highly methylated benzene hydrocarbons, although slow, gave good yields (over 50%) of acids.

Some of the acids, *i.e.*, pyromellitic, benzenepentacarboxylic, and mellitic, were of considerably higher m. p. than previously reported.

In the preparation of benzene-1 : 2 : 3 : 5-tetracarboxylic acid by oxidation of 2 : 4 : 6-trimethylacetophenone, a good yield of 2-carboxyformyltrimesic acid was produced first. This was then oxidised, in poor yield, to the required product.

As part of an attempted preparation of benzene-1 : 2 : 3 : 4-tetracarboxylic acid [8] a dihydronaphthalene-1 : 4-dicarboxylic acid,[9] presumably pure, was dehydrogenated with bromine. Instead of the expected naphthalene-1 : 4-dicarboxylic acid, a brominated product was produced, oxidation of which gave a mixture of approximately equal quantities of phthalic, benzene-1 : 2 : 3 : 4-tetracarboxylic and benzene-1 : 2 : 3 : 5-tetracarboxylic acid, the tetra-acids being inseparable by crystallisation. The formation of benzene-1 : 2 : 3 : 5-tetracarboxylic acid is of significance, since it implies that the treatment of the reaction product from sodium and naphthalene with carbon dioxide yields a dihydronaphthalene-1 : 3-dicarboxylic acid in addition to the 1 : 2- and the 1 : 4-acid previously known to be formed.

EXPERIMENTAL

Chromatography.—Materials used. The "Pyrex"-glass tube (1·8 cm. diam.) was fitted with a tap and sintered-glass disc at the bottom. The height of the tube above the disc should be at least 40 cm.

Silica gel,[11] prepared for chromatography [11] (Messrs. Hopkin and Williams), was used.

Chloroform and *n*-butanol were of analytical quality. Solvent mixtures were prepared by shaking appropriate volumes of *n*-butanol and chloroform with a little distilled water and separating the organic layer.

[6] Resnick, Lee, and Powell, *Analyt. Chem.*, 1955, **27**, 928.
[7] Scott, *ibid.*, p. 367.
[8] Smith and Carlson, *J. Amer. Chem. Soc.*, 1939, **61**, 288.
[9] Walker and Scott, *ibid.*, 1938, **60**, 953.

Procedure. Silica gel (20 g.) and 0·5N-sulphuric acid (10 ml.) are mixed and then slurried with chloroform (80 ml.). The column is packed, at atmospheric pressure, so that the rate of flow is about 3 ml./min.

Acid mixture (up to about 0·1 g.) is dissolved in methanol (0·4 ml.) and diluted with chloroform (0·6 ml.). The solution is added to the top of the column, from a capillary held just above the surface, and allowed to drain into the column. The procedure is repeated twice with washings from the flask, which consist successively of 10% methanol–chloroform (1 ml.) and chloroform (1 ml.).

Chloroform (50 ml.) is added to the column and elution commenced, the eluant being collected in 10-ml. fractions. More solvent is added portionwise (each 50 ml.) before the last 10 ml. of the preceding solvent has drained into the column. The complete range of solvents is chloroform (50 ml.), followed by the following *n*-butanol–chloroform mixtures: 2·5%, 150 ml.; 10%, 100 ml.; 12·5%, 100 ml.; 17·5%, 100 ml.; 25%, 100 ml.; 30%, 100 ml.; 40%, 100 ml.; 50%, 100 ml.; 70%, 200 ml.

With concentrations of alcohol up to about 40%, a flow rate of 2—3 ml./min. can be maintained at atmospheric pressure. At higher concentrations it is necessary to apply slight positive pressure to maintain the rate.

The acid in each fraction is estimated, after addition of water (50 ml.) and a little phenolphthalein, by titration with 0·02N-sodium hydroxide.

Values (ml.), each being the mean of several determinations of peak effluent volumes are as follows: benzoic acid, 20, *iso*phthalic acid, 83, phthalic acid, 108, trimesic acid, 225, trimellitic acid, 254, hemimellitic acid, 359, benzene-1 : 2 : 3 : 5-tetracarboxylic acid, 452, pyromellitic acid, 508, benzene-1 : 2 : 3 : 4-tetracarboxylic acid, 631, benzenepentacarboxylic acid, 808, and mellitic acid, 1040.

Peak effluent volumes were also determined for the two keto-acids, 2-carboxyformyl*iso*phthalic acid, 252, and 2-carboxyformyltrimesic acid, 357. These acids were isolated during attempted preparations of hemimellitic and benzene-1 : 2 : 3 : 5-tetracarboxylic acids, respectively.

Preparation and Isolation of the Acids.—Benzoic and the phthalic acids were obtained commercially. All other acids were prepared in the laboratory.

The hydrocarbon or ketone was oxidised by (generally prolonged) boiling with an excess of aqueous alkaline potassium permanganate. Except for hemimellitic acid, which was purified *via* the barium salt, all the acids were recovered by the following procedure. The excess of permanganate was destroyed and the solution filtered. After acidification with hydrochloric acid the filtrate was evaporated to dryness (water-bath) and the solid residue was extracted with ether, in a Soxhlet apparatus, until all the organic material was removed. The ethereal extract was evaporated to dryness giving the benzene-carboxylic acid which was recrystallised.

Trimesic acid. This acid was obtained from mesitylene (69%; m. p. not determined) and crystallised from 50% aqueous ethanol (Found: C, 51·5; H, 2·5; equiv., 71·7. Calc. for $C_9H_6O_6$: C, 51·4; H, 2·9%; equiv., 70·0).

Trimellitic acid. Oxidation of 2 : 4-dimethylacetophenone gave the acid, m. p. 221—222° (from dilute hydrochloric acid) (Found: C, 50·9; H, 3·1%; equiv., 71·0).

Hemimellitic acid. Oxidation of acenaphthene gave crude 2-carboxyformyl*iso*phthalic acid, which was converted into the *trimethyl ester* (m. p. 144—145°; from ethanol) (Found: C, 55·4; H, 4·2. $C_{13}H_{12}O_7$ requires C, 55·7; H, 4·3%). Subsequent hydrolysis gave the pure *acid*, m. p. 230—231° (Found: C, 45·6; H, 3·7%; equiv., 87·4. $C_{10}H_6O_7,1\frac{1}{2}H_2O$ requires C, 45·3; H, 3·4%; equiv., 87·3).

Hemimellitic acid (40%; m. p. 195—197°) was prepared from naphthalic anhydride, after Graebe and Leonhardt's method[10] (Found: C, 44·3; H, 4·3%; equiv., 81·9. Calc. for $C_9H_6O_6,2H_2O$: C, 43·9; H, 4·1%; equiv., 82·0).

Pyromellitic acid. Oxidation, for 72 hr., of 1 : 2 : 4 : 5-tetramethylbenzene gave pyromellitic acid (61%), m. p. 285—287° (from water) (Found: C, 41·2; H, 3·5%; equiv., 72·0. Calc. for $C_{10}H_6O_8,2H_2O$: C, 41·4; H, 3·5%; equiv., 72·5).

Benzene-1 : 2 : 3 : 5-*tetracarboxylic acid.* Oxidation of 2 : 4 : 6-trimethylacetophenone and crystallisation from dilute hydrochloric acid (1 : 1) gave 2-*carboxyformyltrimesic acid* (91%), m. p. 252° (Found: C, 47·0; H, 2·2%; equiv., 71·3. $C_{11}H_6O_9$ requires C, 46·8; H, 2·1%;

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[10] Graebe and Leonhardt, *Annalen*, 1896, **290**, 218.

[11] Fieser and Peters, *J. Amer. Chem. Soc.*, 1932, **54**, 4347.

equiv., 70·5). The *tetramethyl ester* had m. p. 168—168·5° after crystallisation from ethanol (Found: C, 53·2; H, 4·2. $C_{15}H_{14}O_9$ requires C, 53·3; H, 4·1%).

Prolonged oxidation of the keto-acid gave benzene-1 : 2 : 3 : 5-tetracarboxylic acid (26%), m. p. 246—247° (from dilute hydrochloric acid; 1 : 1) (Found: C, 47·2; H, 2·4%; equiv., 62·7. Calc. for $C_{10}H_6O_8$: C, 47·3; H, 2·4%; equiv., 63·5).

Benzene-1 : 2 : 3 : 4-*tetracarboxylic acid.* β-(3-Acenaphthoyl)propionic acid, m. p. 210·5—212°, was prepared by Fieser and Peters's method.[11] On oxidation, this acid gave benzene-1 : 2 : 3 : 4-tetracarboxylic acid (63%), m. p. 236·5—238° (from dilute hydrochloric acid; 1 : 1) (Found: C, 47·9; H, 2·5%; equiv., 63·8).

Benzenepentacarboxylic acid. The oxidation, for 40 hr., of pentamethylbenzene gave the penta-acid (59%), m. p. 236·5—237° (from dilute hydrochloric acid) (Found: C, 38·5; H, 3·2%; equiv., 68·7. Calc. for $C_{11}H_6O_{10},2\frac{1}{2}H_2O$: C, 38·5; H, 3·2%; equiv., 68·6).

Mellitic acid. 72 Hours' oxidation of hexamethylbenzene gave mellitic acid (99%), m. p. 307° (from hydrochloric acid; 1 : 1) (Found: C, 41·6; H, 1·6%; equiv., 57·2. Calc. for $C_{12}H_6O_{12}$: C, 42·1; H, 1·8%; equiv., 57·0).

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