In connection with investigations in these laboratories into the nucleic acids of normal and cancerous tissues, it has been found desirable to modify the technique to avoid the following disadvantages of the sulfide-spot method: (1) The method will not detect less than 5 micrograms of any given base. (2) It will not distinguish between one base and another, so that a careful consideration of RF values is necessary. (3) Spot-visibility is low and becomes worse with time. Even the darkest spots are barely visible after 24 hours. (4) The use of ammonium sulfide reagent is, understandably, unpleasant.

Silver nitrate has already been used to precipitate purines in the separation of these compounds from purine nucleosides.[11] We have now developed a visualization procedure utilizing silver nitrate as follows: (a) The mixture of purine and pyrimidine bases[12] in dilute acid solution (*e. g.*, 5% trichloroacetic acid) is resolved in the usual chromatographic manner, using water-saturated butyl alcohol as the moving solvent.[3] The chromatogram is allowed to dry. (b) A 2% solution of silver nitrate is allowed to trickle from a pipet over the suspended chromatogram. (c) After draining about 5 minutes, the chromatogram is passed through a bath of 0.5% sodium dichromate. The entire sheet is thus covered with a red silver chromate precipitate. (d) The chromatogram is then transferred immediately to a 0.5 *N* nitric acid bath, in which the silver chromate slowly dissolves. Left behind are less soluble red deposits (presumably a purine/silver chromate complex) at the positions occupied by adenine and guanine. (e) The chromatogram is removed from the nitric acid bath *while the background is still slightly pink*, and then washed with water. It should be noted that over-exposure of the chromatogram to acid in step (d) will lead to the leaching out of the purine complex as well. The guanine chromate-spot is the more sensitive to the nitric acid, so that it is usually smaller than the adenine chromate-spot and shows a greater tendency to fade with time.

If the characteristics of the chromate-spots and the sulfide-spots are compared, the following points may be noted: (1) The chromate-spot is detectable when as little as 0.5 microgram of purine is used. It is thus a full order of magnitude more sensitive than is the sulfide-spot. (2) The chromate-spot is specific for purines and does not form with cytosine,[13] thymine or uracil. Since the RF values for adenine and guanine are quite different, identification of the spots is thus possible at a glance. (3) The chromate-spots are brilliantly red, sharply defined, and highly visible. The adenine spot, particularly, is only slightly subject to fading and can be kept as a semi-permanent record. (4) In preparing the chromate-spot, the use of ammonium sulfide is avoided.

(11) Kerr and Seraidarian, *J. Biol. Chem.*, **159**, 211 (1945).

(12) Only those bases occurring naturally in nucleic acids, *i. e.*, adenine, guanine, cytosine, thymine, and uracil, are here considered.

(13) A sample of cytosine was made available to us through the courtesy of Dr. Elkan R. Blout of Polaroid Corporation, Cambridge, Massachusetts.

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## Paper Partition Chromatography of Some Simple Phenols[1]

BY RICHARD F. RILEY[2]

It was desirable to apply the technique of partition chromatography to the identification of degradation products of a phenyl ether derived metabolically from Myanesin (3-(*o*-toloxy)-1,2-propanediol).[3] The application of this technique to the separation of common phenols has very recently been reported.[4] Our work, carried out prior to the appearance of the earlier publication, is presented as our methods differ considerably and likewise gave satisfactory results.

### Experimental

The one dimensional descending boundary technique was employed throughout.[5] Solutions of phenols for examination were applied to Whatman no. 1 filter paper as micro drops to give ~0.5 cm. spots containing ~20 μg. of compound.

The various phenols were visualized by spraying the dried developed chromatograms with a 2% aqueous solution of phosphomolybdic acid and then exposing the moist strips to ammonia vapor. Di- and trihydroxy phenols containing —OH groups *ortho* or *para* to each other appear immediately as well-defined dark blue spots which darken on exposure to ammonia vapor. Simple phenols and polyhydroxy phenols in which —OH groups are in *meta* positions appear as spots shading from blue to green after exposing the sprayed chromatogram to ammonia vapor. Other reagents were also tested: ammoniacal silver reagent[6] will make visible *ortho* and *para* dihydroxy phenols; resorcinol and derivatives give red spots after spraying with 2% fructose and heating carefully in the presence of hydrochloric acid. Sprays of 1 to 2% aqueous or alcoholic ferric chloride may be useful in some instances; however, for the phenols listed, the phosphomolybdic acid reagent appeared to be more sensitive and general than the other reagents mentioned.

**Results.**—A summary of $R_f$ values determined under our operating conditions for several phenols in several solvent combinations is given in Table I.

Amyl alcohol–water achieves a separation of mono, di- and tri-hydroxyl phenols although resolution of members within a group is not particularly satisfactory. Addition of benzene to alcohol–water systems was, in general, found to increase the dispersion, though decreasing the $R_f$ values at the same time so as to give rather incomplete resolution. However, by allowing the

(1) Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

(2) University of California Atomic Energy Project, Post Office Box 4164, Los Angeles 24, California.

(3) R. F. Riley, THIS JOURNAL, **72**, 5712 (1950).

(4) R. A. Evans, W. H. Parr and W. C. Evans, *Nature*, **164**, 574 (1949).

(5) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

(6) To 0.1 *N* $AgNO_3$ solution add concentrated ammonium hydroxide dropwise until the precipitate formed just redissolves.

TABLE I

$R_f$ VALUES OF SOME COMMON PHENOLS IN VARIOUS SOLVENT SYSTEMS

The solvent systems were prepared by equilibrating at room temperature (approximately 23°) the proportions by volume of the solvents indicated. The organic phase was employed as the descending solvent and the aqueous phase was placed within the chamber to maintain equilibrium conditions.

| Phenol | Solvent systems: *n*-$C_5H_{11}OH$–$H_2O$ (mutually saturated) | *n*-$C_4H_9OH$–$C_6H_6$–$H_2O$ 1:9::10 | *n*-$C_4H_9OH$–$C_6H_6$–$H_2O$ 1:19::20 |
|---|---|---|---|
| Hydroquinone | 0.80 | 0.43 | 0.078 |
| Resorcinol | .83 | .54 | .13 |
| Catechol | .86 | .70 | .38 |
| Toluhydroquinone | .85 | .. | .23 |
| Phloroglucinol | .61 | .. | .00 |
| Pyrogallol | .65 | .. | .025 |
| α-Naphthol | .90 | .. | .83 |
| β-Naphthol | .92 | .. | .85 |

descending solvent face of the alcohol–benzene–water system to drip from the lower boundary of the paper, greater resolution was obtained in some instances. For example, with butyl alcohol–benzene–water (1:19:20), it was found possible to resolve completely a mixture of the first six phenols listed in the table. By continuing development until the most rapidly moving component (catechol) was about to pass from the paper, the following relative rates of migration were found: (hydroquinone = 1.00), phloroglucinol, 0.00; pyrogallol, 0.33; resorcinol, 1.45; toluhydroquinone, 2.4; catechol, 4.7.

The following systems were tested with the listed phenols but found unsatisfactory because of poor resolution: cyclohexanone–water, hexane–acetonitrile, chloroform–formamide and benzene–formamide.[7] Butyl alcohol–water effected some resolution as did benzene–water but these systems were comparatively unsatisfactory for the compounds listed.

A serious limitation of the method may be the volatility of phenols of interest. For example, *o*-cresol completely evaporates during the interval necessary for the development and drying of the chromatogram.

**Acknowledgment.**—The author wishes to thank Dr. Alejandro Zaffaroni for his helpful advice.

(7) A. C. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950).

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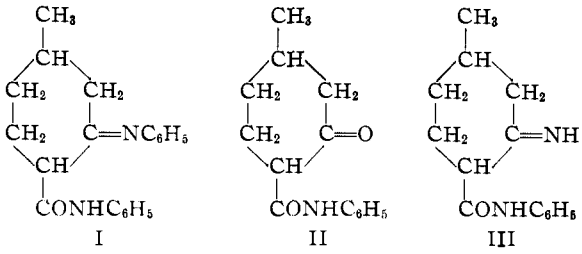
## 2-Keto-4-methylcyclohexanecarboxyanilide

BY ROYSTON M. ROBERTS AND MARVIN B. EDWARDS

Kötz and Merkel[1] reported the preparation of the "anilinoanilid," I, by heating ethyl 2-keto-4-methylcyclohexanecarboxylate with aniline. In connection with other work,[2] we attempted to repeat their preparation We obtained a product with the reported melting point, but the analysis corresponded to the ketoanilide, II. It was converted to the corresponding iminoanilide, III, which reacted with aniline to evolve ammonia, as expected.[3] The product of this reaction, presumably I, was not isolated in a pure state. These results are compatible with the results of Sen and Basu[4] who obtained analogs of II, but were unable to obtain any analogs of I from ethyl 2-ketocyclohexanecarboxylate, and with the work of Thomson and Wilson,[3] who prepared analogs of I under different conditions. Hence, it would appear that the analytical data on the compound reported by Kötz and Merkel was in error.



I II III

### Experimental[5]

**2-Keto-4-methylcyclohexanecarboxyanilide** (II).—Equimolar amounts of ethyl 2-keto-4-methylcyclohexanecarboxylate and aniline were heated at 140° for 2 hours. The crude product was obtained in 24% yield, m. p. 123–127°. Recrystallizations from benzene–petroleum ether and ethanol gave a pure material, m. p. 129–130°.

*Anal.* Calcd. for $C_{14}H_{17}O_2N$: C, 72.70; H, 7.42. Found: C, 72.73; H, 7.36.

**2-Imino-4-methylcyclohexanecarboxyanilide** (III).—This compound was prepared according to Thomson and Wilson[3] and recrystallized from ammoniacal ethanol: m. p. 128–129°; m. p. of mixture with the above compound, 98–108°.

*Anal.* Calcd. for $C_{14}H_{18}ON_2$: C, 73.01; H, 7.88; N, 12.17. Found: C, 72.61; H, 7.55; N, 12.09.

When this compound was heated with aniline in petroleum ether (b. p. 100–140°), ammonia was evolved over a period of several hours. The oil which separated on cooling was difficult to crystallize. A sample which melted at 95–99° was obtained, but further attempts to purify it resulted in hydrolysis to compound II.

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(2) R. M. Roberts and M. B. Edwards, THIS JOURNAL, **72**, 5537 (1950).

(3) J. K. Thomson and F. J. Wilson, *J. Chem. Soc.*, 111 (1935).

(4) H. K. Sen and U. Basu, *J. Ind. Chem. Soc.*, **6**, 309 (1929).

(5) Microanalyses by Clark Microanalytical Laboratory, Urbana, Ill.

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## The Osmotic and Activity Coefficients of Cobalt Bromide and Cobalt Iodide

BY R. A. ROBINSON, H. J. MCCOACH AND C. K. LIM

Osmotic and activity coefficients of a high order of accuracy have been obtained for calcium chlo-