THE PHENOLIC ACIDS OF HUMAN URINE*

PAPER CHROMATOGRAPHY OF PHENOLIC ACIDS

By MARVIN D. ARMSTRONG, KENNETH N. F. SHAW, AND PATRICIA E. WALL

(From the Laboratory for the Study of Hereditary and Metabolic Disorders and the Departments of Biological Chemistry and Medicine, University of Utah College of Medicine, Salt Lake City, Utah)

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In the course of an investigation of the metabolism of aromatic compounds in phenylketonuria, it was found that, along with the abnormal metabolism of phenylalanine in this disease, there are other striking deviations from normal. These are manifested by the excretion of greatly increased amounts of some indole acids (1) and phenolic acids (2, 3) which are present in normal human urine in small quantities. In order to gain a clearer understanding of the deranged metabolism of aromatic compounds in phenylketonuria and in other disorders, it became necessary to examine the phenolic constituents of normal human urine.

Some procedures already have been reported for the chromatographic characterization of various phenolic acids (4–9). The compounds for which detailed information has been reported are few in number, and there has been no systematic investigation of the simple compounds which might be expected to occur in urine. The purpose of this paper is to report a description of the phenolic acids of human urine. A combination of solvent systems which is suitable for the separation of forty-nine authentic aromatic acids by two-dimensional paper chromatography has been developed.

EXPERIMENTAL

Some of the compounds in these experiments were obtained from commercial sources. Others were prepared by methods recorded in the literature or were made by unequivocal syntheses when they had not been reported previously. Commercially available solvents of the best grade which could be purchased were used without further purification.

5 γ samples of the compounds were satisfactory for testing their behavior on chromatograms; larger amounts did not show significantly different behavior in the solvent systems reported here (Table I). When chromatograms were developed with benzene-propionic acid-water, the paper sheets

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were allowed to equilibrate in the chambers for 1 hour before development was commenced. No preliminary equilibration was employed with the other solvent systems. Fresh solvents were used for each chromatogram. This is critical with the benzene-propionic acid-water system, since enough of the water in the mixture is absorbed preferentially by paper to change the composition of the mixture and to cause a marked disturbance of the movement of compounds in the system if the solvent is used a second time. The *n*-butanol-acetic acid-water system was prepared freshly for each run, as suggested by Berry *et al.* (10); R_F values were not affected markedly if the solvent was allowed to age, however. It was necessary to apply weights to the covers of chambers containing the isopropyl alcohol-ammonia-water system; otherwise sufficient ammonia escaped during an overnight run to cause variations in R_F.[1]

Chromatograms were developed at 25° by capillary ascent on Whatman No. 1 filter paper; the solvent fronts were allowed to advance 20 to 25 cm. Chromatograms were examined under ultraviolet light (Mineralight) for the ability of compounds to fluoresce or to quench the background fluorescence of the paper. The phenolic acids were visualized by coupling them with diazotized sulfanilic acid as described by Berry *et al.* (10) and with diazotized *p*-nitroaniline (4). Reducing phenols and aromatic keto acids were detected with ammoniacal silver nitrate (10), and aroylglycines with the hippuric acid reagent of Gaffney *et al.* (11). The Folin-Ciocalteu reagent (12) could be used to detect phenolic acids, but the lack of a characteristic color with individual compounds made this reagent of little use in comparison with the diazotized amines.

When chromatograms have been developed in the benzene-propionic acid or butanol-acetic acid system they should be allowed to stand overnight or should be heated in an oven at 100° for 3 minutes before they are sprayed with the diazotized amines. If this is not done, the residual acid present on the paper interferes with the development of the characteristically colored dyes. It is essential that great care be taken to protect these chromatograms from contamination with vapors of phenol which may be present in many laboratories, particularly if phenolic systems are being used for other chromatographic work. Otherwise, background color develops and conceals the presence of many compounds which may be present in small amounts.

The chromatographic behavior of the authentic compounds is listed in Table I. The most satisfactory combination of systems for two-dimen-

[1] Differences may be noted in R_F values obtained by workers in other laboratories when conditions reported here are followed exactly. The low relative humidity (20 to 60 per cent) and atmospheric pressure (640 mm. of Hg) at the altitude of this laboratory (5000 feet) undoubtedly should lead to differences in the observed R_F, but the relative values of different compounds should be the same.

sional chromatography has proved to be isopropyl alcohol-aqueous ammonia-water (8:1:1) for the first run, followed by benzene-propionic acid-water (2:2:1, organic phase) as the second system. Satisfactory results are not obtained if the order in which the systems are used is reversed. The location of the known substances on the two-dimensional chromatograms may be estimated from the R_F values listed in Table I. A few substances show slightly altered behavior when chromatographed in the mixture found in urine, but the tentative identification of each has been confirmed by the behavior of authentic material added to urine extracts.

Examination of Phenolic Acids of Urine—An inspection of the chromatographic behavior of the authentic phenolic acids studied here, as revealed by the R_F values of Table I, shows that one-dimensional chromatography is of little use in examining a complex mixture such as that presented by the phenolic acids of urine and that a two-dimensional system is essential. It is necessary to separate and concentrate the acids by extraction into an organic solvent, since chromatography of urine itself gives little indication of their presence; they are present in small amounts, and other compounds interfere with their chromatographic characteristics. In order to compare the excretion of the compounds by different individuals and by the same person at different times, the amount of extract chromatographed is based upon the creatinine content of the urine sample extracted. An assay of the preformed creatinine content of a sample is made, and, when the final organic extract is prepared, it is diluted to correspond to a definite amount of creatinine in the original sample. With samples containing at least 100 mg. of creatinine, an extract can conveniently be prepared in a concentration such that 1 ml. corresponds to 10 mg. of creatinine in the original sample. With smaller samples of urine, such as are obtained from infants, a final concentration of 1 ml. per mg. of creatinine can usually be prepared.

Other investigators routinely have subjected urine samples to acid hydrolysis before extraction (4, 7, 8). Only a small proportion of the organic acids in human urine has proved to be conjugated in forms which do not extract into organic solvents; on the other hand, some of the urinary compounds are sensitive to acid. Unhydrolyzed urine has been used for all of the present work, and care has been taken to avoid the decomposition of compounds which might be unstable to acid conditions. In order to minimize decomposition of unstable compounds, batch extractions have been carried out rather than continuous extractions. For routine purposes, 3 × 0.5 volume of portions of ethyl acetate[2] have been used to ex-

[2] (*a*) The solubility of many of the polar phenolic acids is more favorable in ethyl acetate than in ether, (*b*) ethyl acetate does not form peroxides which might oxidize sensitive compounds, and (*c*) for routine use in the laboratory the fire hazard with ether would be considerable.

TABLE I

Chromatographic Behavior of Phenolic Acids

Solvent systems, Ipr-NH_3, isopropyl alcohol-aqueous ammonia-H_2O, 8:1:1; Bz-Prop acid, benzene-propionic acid-H_2O, 2:2:1 (organic phase); aq. KCl, 20 per cent aqueous KCl; Bu-Ac acid, *n*-butanol-acetic acid-H_2O, 8:2:2; Bu-Pyr-Diox, *n*-butanol-pyridine-dioxane-H_2O, 70:20:5:5. *Reagents*, DzSA, diazotized sulfanilic acid (10); DzPNA, diazotized *p*-nitroaniline (4). *Abbreviations*, lt., light; dk., dark; R, red; O, orange; Y, yellow; Bl, blue; P, purple; G, gray; Br, brown; Bk, black.

Acid	R_F					Qualitative color reactions			
	Ipr-NH_3	Bz-Prop acid	Aq. KCl	Bu-Ac acid	Bu-Pyr Diox-	Ultra-violet light	DzSA	DzPNA	Mis-cella-neous
o-Hydroxy-benzoic	0.78	0.88	0.66	0.90	0.43	Bl	Lt. Y	Lt. R	
hippuric	0.22	0.43	0.68	0.86	0.30	"	" "	" "	O*
phenyl-acetic	0.76	0.57	0.86	0.91	0.49		Dk. O	Dk. P	
mandelic (*a*)†	0.58	0.13	0.82	0.54	0.30	Dark	Y	Lt. R	
	(0.68)		(0.88)	(0.68)					
phenylpro-pionic	0.70	0.74	0.77	0.91	0.72		O	Dk. P	
cinnamic	0.35	0.70	0.44	0.93	0.75	White	Lt. O	" "	
phenyl-lactic	0.59	0.29	0.84	0.85	0.28		" "	Lt. "	
phenyl-pyruvic (*b*)†	‡	0.21	0.85	0.70	0.43	White	O	P	G§
phenyl-pyruvic (lactone)	0.54	0.88	0.44	0.85	0.87		Lt. O	Lt. Br	
m-Hydroxy-benzoic	0.39	0.53	0.70	0.88	0.50	Dk. Bl	Dk. Y	Dk. R	
hippuric	0.33	0.09	0.75	0.78	0.19	" "	" "	" "	O*
phenyl-acetic	0.46	0.49	0.83	0.92	0.46		Lt. O	Lt. P	
mandelic	0.37	0.07	0.87	0.68	0.19		Dk. Y	Dk. R	
phenylpro-pionic	0.54	0.61	0.77	0.92	0.68		Lt. O	Lt. P	
cinnamic	0.44	0.58	0.39	0.93	0.60		Dk. Y	Dk. R	
phenyl-lactic	0.51	0.12	0.86	0.82	0.21		Lt. O	Lt. P	
phenyl-pyruvic (*c*)†	‡	0.13	Streak	0.74	0.50‡	Bl	Dk. Y → Br	G	Br§
p-Hydroxy-benzoic	0.23	0.55	0.54	0.87	0.68	Dark	Dk. Y	R	
hippuric	0.15	0.07	0.72	0.78	0.18	"	Lt. O	Lt. R	O*
phenyl-acetic	0.42	0.49	0.82	0.92	0.50		P	Bl-P	
mandelic	0.33	0.07	0.87	0.68	0.16		Dk. Y	R	

TABLE I—*Continued*

Acid	R_F					Qualitative color reactions			
	Ipr-NH_3	Bz-Prop acid	Aq. KCl	Bu-Ac acid	Bu-Pyr-Diox	Ultra-violet light	DzSA	DzPNA	Miscellaneous
p-Hydroxyphenylpropionic	0.51	0.61	0.73	0.93	0.69		P	P	
cinnamic	0.28	0.58	0.31	0.93	0.67	Dark	R	Bl-G	
phenyllactic	0.45	0.13	0.85	0.82	0.19		P	P	
phenylpyruvic†	‡	0.15	Streak	0.78	‡		O-R	Dk. G	G§
3-MeO-4-OH-benzoic	0.22	0.80	0.66	0.89	0.60	White	R-O	" P	
hippuric	0.16	0.18	0.66	0.74	0.16		Lt. O-R	P	O*
phenylacetic	0.39	0.66	0.82	0.88	Streak	Lt. Bl	" R	G-Br	G§
mandelic	0.28	0.16	0.84	0.71	0.20		O → R	Bl-G	"
phenylpropionic	0.43	0.81	0.74	0.91	0.65		R-P	G	Br§
cinnamic	0.27	0.80	0.35	0.88	0.63	Bl	P (fades)	Bl-G	
phenylpyruvic	‡	0.25	Streak	0.73	‡	"	O-R	G	G-Br§
p-Hydroxycinnamoylglycine	0.23	0.09	0.46	0.80	0.20	Dk. Bl	Dk. R	Bl-G	O*
Feruloylglycine	0.18	0.19	0.35	0.75	0.18	Bl	P (fades)	"	"
Protocatechuic	0.06	0.16	0.73	0.83	Streak	Dark	Lt. Br	Br	Bk§
Gentisic	0.68	0.26	0.53	0.89	0.34	Bl	White	Lt. Y	"
α-Resorcylic	0.24	0.09	0.64	0.78	0.39	Dk. Bl	Br-O	Br	
β-Resorcylic	0.39	0.38	0.48	0.92	0.34	Bl	Dk. Br	Dk. Br	
γ-Resorcylic	0.77	0.11	0.50	0.54	0.48	Dark	" "	Lt. "	
Gallic	0.01‡	0.02	Streak	0.60	0.29‡	"	Br	Dk. "	Bk§
Syringic	0.18	0.79	0.57	0.87	‡		R (fades)	Y	G§
Homoprotocatechuic	0.06‡	0.15	0.75	0.76	Streak	Dark	Lt. Br	Br	Bk§
Homogentisic	0.05‡	0.09	0.90	0.75	0.34		" "	"	"
Caffeic	0.05‡	0.19	0.36	0.78	0.53	Bl	" "	"	"
Kynurenic	0.52	0.16	0.37	0.55	0.21	"			
Xanthurenic	0.08	0.03	0.24	0.53	0.23	"	Dk. R	P	
3-Hydroxyanthranilic	0.12‡	0.38	Streak	0.85	0.65	"	Lt. Br	G	Bk§
5-Hydroxyindoleacetic	0.32	0.16	0.58	0.76	0.45	Faint pink	Dk. R	G	Bl‖ Bk§

TABLE I—*Concluded*

* *p*-Dimethylaminobenzaldehyde in acetic anhydride (11).

† (*a*) *o*-Hydroxymandelic acid gives two yellow spots in many of the solvent systems. One of these perhaps represents the lactone and one the acid. (*b*) *o*-Hydroxyphenylpyruvic acid was used in the form of its lactone, 3-hydroxycoumarin. Experiments in which the lactone was allowed to stand in the presence of excess 0.1 N NaOH and then neutralized before applying to chromatograms showed the presence of other components. One of these, in some systems, showed properties expected for the free pyruvic acid and the R_F values for it are listed. (*c*) The pyruvic acids bore evidence of decomposition and the presence of several components in many systems. This behavior perhaps occurs as a result of the keto-enol tautomerism of these compounds and, in addition, of their reactivity. Special conditions will be necessary for the chromatography of these compounds.

‡ Decomposes.

§ Ammoniacal $AgNO_3$ (10).

‖ *p*-Dimethylaminobenzaldehyde in aqueous HCl (10).

tract urine which was acidified to pH 1 to 2 and saturated with sodium chloride. Reextraction of samples that had been subjected to this treatment showed that small amounts of the more polar compounds are not completely extracted. This standard procedure has been used routinely for the examination of normal and of pathological urines, however, and marked variations in the amounts of polar metabolites present in different samples are evident, even though the extraction of some of the compounds is not quantitative.

Procedure by Which Phenolic Acids of Urine Are Prepared for Chromatography—The volume of a sample of urine is measured and the preformed creatinine is estimated with the Jaffe procedure. An aliquot corresponding to a definite amount of creatinine is diluted so that it contains 0.5 to 0.8 mg. of creatinine per ml. The diluted urine is chilled in an ice bath, acidified to pH 1 to 2 (indicator paper) by the careful addition of concentrated HCl, and saturated with NaCl (approximately 26 gm. per 100 ml.). It is then extracted with three successive 0.5 volume portions of ethyl acetate. The first extraction is frequently made difficult by the formation of an emulsion; this may be broken by separating as much as possible of the clear portions of the aqueous and organic layers and centrifuging the residual emulsion. The second and third extractions with ethyl acetate usually proceed smoothly. The combined ethyl acetate solutions are extracted by *prolonged* and thorough shaking with small portions of 10 per cent $NaHCO_3$ solution. An initial extraction with an amount such that 1 ml. corresponds to about 20 mg. of creatinine in the original sample of urine, followed by two more extractions with half that volume for each extraction, usually proves satisfactory. The combined bicarbonate extracts are chilled in an ice bath, acidified to pH 1 to 2 by the addition of concentrated HCl, saturated with salt, and then extracted with successive

small portions of ethyl acetate. The ethyl acetate extracts may be collected in a graduated cylinder of an appropriate size, and the final volume of ethyl acetate may then be adjusted to correspond to a definite amount

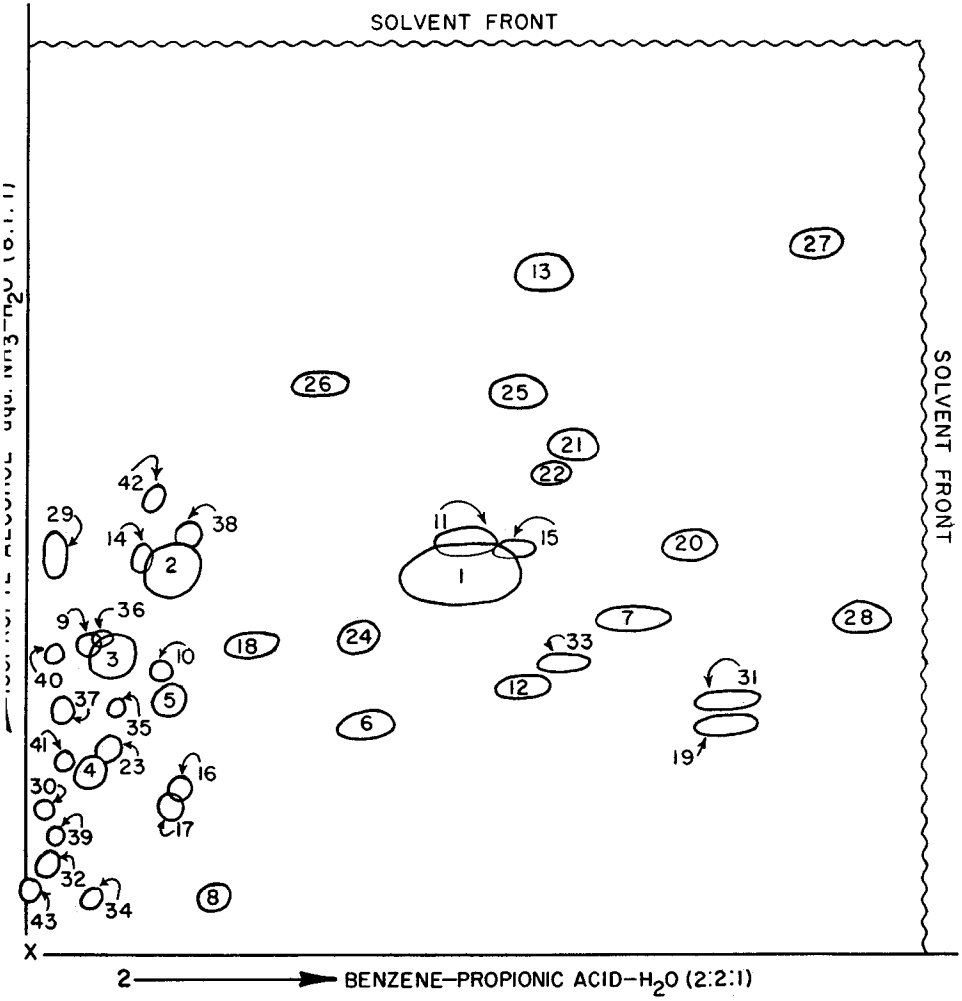

FIG. 1. Phenolic acids in human urine. The numbers refer to the compounds listed in Table II.

of creatinine in the original urine. When it is desired to store extracts, they may be dried by adding anhydrous Na_2SO_4 and decanting the ethyl acetate into a container; the extract may then be stored in a deep freeze at $-15°$.[3]

[3] Used penicillin vials are excellent containers for storing extracts.

TABLE II

Phenolic Acids in Human Urine

Compound No.	Description	R_F	
		Ipr-NH_3	Bz-Prop acid
1	*p*-Hydroxyphenylacetic acid	0.42	0.49
2	Dark yellow (DzSA); light purple (DzPNA)	0.43	0.16
3*	*m*-Hydroxyhippuric acid	0.33	0.09
4*	*p*-Hydroxyhippuric acid	0.20 (0.15)†	0.07
5	5-Hydroxyindoleacetic acid	0.28	0.16
6*	Salicyluric acid	0.25	0.38
7*	Homovanillic acid	0.37	0.68
8	Dark red (DzSA); gray (DzPNA)	0.06	0.21
9*	*p*-Hydroxymandelic acid	0.34	0.07
10	Orange → red (DzSA); blue (DzPNA)	0.31	0.14
11	*m*-Hydroxyphenylacetic acid	0.45	0.49
12	*p*-Hydroxybenzoic acid	0.29 (0.23)†	0.55
13	*o*-Hydroxyphenylacetic acid	0.75	0.58
14	*p*-Hydroxyphenyllactic "	0.44	0.13
15	*m*-Hydroxybenzoic acid	0.45 (0.39)†	0.47
16*	Feruloylglycine	0.18	0.17
17*	Vanilloylglycine	0.16	0.16
18	Orange-red (DzSA); blue-gray (DzPNA)	0.34	0.25
19	Vanillic acid	0.25	0.78
20	Dihydroferulic acid	0.45	0.74
21*	*m*-Hydroxyphenylpropionic acid	0.56	0.61
22	*p*-Hydroxyphenylpropionic acid	0.53	0.59
23*	α-Resorcylic acid	0.22	0.09
24	Dark yellow (DzSA); red (DzPNA)	0.35	0.37
25	" " " reddish purple (DzPNA)	0.62	0.55
26	Reddish orange (DzSA); light purple (DzPNA)	0.63	0.33
27	Salicylic acid	0.78	0.88
28	Red (DzSA); blue (DzPNA)	0.37	0.93
29	Orange → gray (DzSA); light brown (DzPNA) (pregnancy urine)	0.44	0.03
30	Dark brown (DzSA); purple-brown (DzPNA)	0.16	0.02
31	Ferulic acid	0.29	0.78
32	Dark yellow (DzSA); light brown (DzPNA)	0.10	0.02
33	*p*-Hydroxycinnamic acid	0.33	0.60
34	Purple (DzSA); blue-purple (DzPNA)	0.06	0.07
35*	*p*-Hydroxycinnamoylglycine	0.27	0.10
36	Orange (DzSA); yellow (DzPNA)	0.34	0.07
37	Reddish purple (fades) (DzSA); gray (DzPNA)	0.27	0.04
38	Purple (DzSA); blue-purple (DzPNA)	0.46	0.18
39	Maroon (DzSA); purple (DzPNA)	0.13	0.03
40	Gray (DzSA); blue-gray (DzPNA)	0.33	0.03
41	Light orange-red (DzSA); purple (DzPNA)	0.21	0.04
42	Purple (DzSA); purple (DzPNA)	0.50	0.14
43	Brown (DzSA); gray (DzPNA)	0.07	0.00

TABLE II—*Concluded*

* Compounds not previously reported to be normal constituents of urine.

† Compounds in which R_F in urine extracts differs from R_F when measured alone. The values in parentheses are those obtained with the pure compound alone.

For chromatography, an amount of the ethyl acetate solution corresponding to 1 mg. of creatinine in the original urine has usually proved satisfactory. Larger quantities may be chromatographed when samples from individuals who have been eating synthetic diets are prepared, but urine from normal persons on a natural diet usually contains so much hippuric acid that the chromatography of other compounds is disturbed. With urine from patients with phenylketonuria, the presence of large amounts of phenylpyruvic and phenyllactic acids and phenylacetylglutamine makes it impractical to use a sample larger than one corresponding to 0.5 mg. of creatinine. The extracts are applied to paper from a micro pipette with a stream of air at room temperature directed at the paper to aid the evaporation of the solvent. The diameter of the spot of the acids deposited for chromatography should not exceed 7 mm. An overnight run (16 hours) at 25° is required for a 25 cm. solvent advance with the isopropyl alcohol-ammonia system and a 3 hour run with the benzene-propionic acid system.

Fig. 1 is a schematic diagram representing the location of the well defined phenolic acids which are found in most samples of normal human urine. Table II provides a list of these compounds. When the location on the chromatogram and the characteristic reactions correspond to those of known compounds, the material is given a tentative identification. Materials which do not correspond to any of the standard compounds are described by their characteristic color with the diazotized amines. The compounds have been arbitrarily designated by number. When an amount of extract corresponding to 1 mg. of creatinine is chromatographed, Compounds 1 to 10 may be detected in almost all samples of urine in amounts decreasing with increased number. Compounds 11 to 17 may be detected in a majority of the samples, and the remainder of the compounds described is present in only an occasional sample of urine or is present in much smaller amounts.[4]

It should be noted that the alkaline medium used for the first solvent in the chromatography reported here leads to the decomposition of many easily oxidized dihydroxyphenyl compounds. Further work with other

[4] The amounts of the acids excreted daily varied greatly under different circumstances. As a rough approximation, it may be estimated that for most individuals under average conditions the amounts of Compounds 1 to 10 excreted daily range from 20 to 25 mg. down to about 2 mg., and of Compounds 11 to 17 from 5 mg. to less than 0.5 mg.

systems will be necessary to characterize these compounds, among which would be homogentisic, homoprotocatechuic, and caffeic acids, as well as *o*- and *p*-hydroxy- and 3-methoxy-4-hydroxyphenylpyruvic acids.

DISCUSSION

Two-dimensional paper chromatography of the phenolic acids of human urine reveals a complex mixture of closely related compounds. Approximately 400 normal and pathological samples have been examined; the compounds definitely characterized in this paper are those which have been found to occur in all or at least several samples. Many more phenolic acids have been observed in the course of this work, but are not reported, either because they were observed in only a single sample of urine or because they gave ill defined spots on the chromatograms. In addition, some substances are not reported which have been observed in small amounts and which are apparent only transiently on the freshly sprayed chromatograms.

The preliminary and tentative identification made when the qualitative reactions and chromatographic behavior of a compound in urine extracts correspond to authentic compounds is probably correct. It should be emphasized, however, that final identification must be based upon actual isolation and comparison with the authentic substance. A great many of these compounds are closely related chemically and have similar solubility behavior and qualitative reactions.

The variety of phenolic acids observed in these experiments is of interest because of their number and their nature. Of the twenty-three acids accorded a tentative identification, ten have not been previously reported to occur as normal constituents of urine. Two series of compounds, those containing an *m*-hydroxyphenyl group (Compounds 2, 3, 11, 15, 21) (13) and the 3-methoxy-4-hydroxyphenyl group (Compounds 7, 16, 17, 19, 20, 31) are of particular interest, since a wide-spread metabolic occurrence of them had not been previously suspected.

When the conclusive identification of each compound has been made, it will be important to establish whether it is an end-product of endogenous metabolism, or whether it occurs as a result of the action of intestinal microorganisms or is derived from food constituents. When these factors have been established, it will be possible to examine alterations in the excretion of these phenolic acids that have been observed in phenylketonuria and in some other pathological conditions in an attempt to gain a better understanding of the relation of the abnormal urinary compounds to the pathological state.

SUMMARY

The chromatographic behavior of forty-nine simple phenolic acids is described. The chromatographic properties of forty-three distinctive

phenolic acids that have been observed in samples of urine from 400 individuals, both healthy and ill, are described, and a preliminary identification is given for twenty-three of them.

BIBLIOGRAPHY

1. Armstrong, M. D., and Robinson, K. S., *Arch. Biochem. and Biophys.*, **52**, 287 (1954).
2. Armstrong, M. D., and Robinson, K. S., *Federation Proc.*, **13**, 175 (1954).
3. Armstrong, M. D., Shaw, K. N. F., and Robinson, K. S., *J. Biol. Chem.*, **213**, 797 (1955).
4. Bray, H. G., Thorpe, W. V., and White, K., *Biochem. J.*, **46**, 271 (1950).
5. Swain, T., *Biochem. J.*, **53**, 200 (1953).
6. Boscott, R. J., and Bickel, H., *Scand. J. Clin. and Lab. Invest.*, **5**, 380 (1953).
7. Boyland, E., Manson, D., Solomon, J. B., and Wiltshire, G. H., *Biochem. J.*, **53**, 420 (1953).
8. Boscott, R. J., and Cooke, W. T., *Quart. J. Med.*, **23**, 307 (1954).
9. Dalgliesh, C. E., *J. Clin. Path.*, **8**, 73 (1955).
10. Berry, H. K., Sutton, H. E., Cain, L., and Berry, J. S., *Univ. Texas Pub., No. 5109*, 22 (1951).
11. Gaffney, G. W., Schreier, K., DiFerrante, N., and Altman, K. I., *J. Biol. Chem.*, **206**, 695 (1954).
12. Folin, O., and Ciocalteu, V., *J. Biol. Chem.*, **73**, 627 (1927).
13. Armstrong, M. D., Shaw, K. N. F., and Wall, P. E., *Federation Proc.*, **14**, 174 (1955).

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