

Preparation and properties of some compounds of biological interest

In connection with work on the identification of unknown urinary metabolites, a number of new compounds were synthesized. This communication describes the preparation and chromatographic characteristics of thirteen such compounds.

Preparation of materials

Catechol monosulfate. One drop of chlorosulfonic acid was stirred vigorously with 0.1 g of catechol, dissolved in 2 ml of benzene, for 5 min at 0°. 5 ml of ice water were added and the mixture extracted three times with 10 ml portions of ether to remove unreacted catechol.

The aqueous layer was chromatographed in BuOH-AcOH-H₂O (4:1:1). Two bands were identified by spraying a strip with diazotized sulfanilic acid (DSA). The upper band was catechol monosulfate, and the lower was probably catechol disulfate. The area corresponding to catechol monosulfate was cut out and eluted with water.

Catechol monophosphate. The method of GENVRESSE¹ was modified as follows: Catechol (1.1 g, 0.01 mole) was heated with 1.43 g of P₂O₅ (0.01 mole) *in vacuo* (15 mm Hg) at 120-130° for 2 h, then distilled at 200° to remove excess catechol. The residue was dissolved in water, and chromatographed in BuOH-AcOH-H₂O (4:1:1). The area containing catechol monophosphate was cut out and eluted with water.

4-Guanidinoimidazole. Guanine was heated with 4 N hydrochloric acid for 2 h at 160° in a sealed tube². The reaction mixture, after chromatographic separation in isopropanol-concentrated ammonium hydroxide-water (8:1:1), gave two DSA-positive spots, one yellow and the other red, changing to mauve. The yellow spot was xanthine and the other was guanidinoimidazole, which was confirmed by a positive pentacyanoaquaferriate (PCF) reaction.

4-Guanidino-5-imidazolecarboxamide. 4-Amino-5-imidazolecarboxamide (AIC) was heated with cyanamide in a boiling water bath for 1 h. The unreacted AIC was removed by chromatographing the mixture in isopropanol-ammonium hydroxide-water (8:1:1). 4-Guanidino-5-imidazolecarboxamide gave a positive PCF test in addition to a positive DSA.

Methyl 4-aminoimidazole-5-carboxylate. This was prepared by the catalytic reduction of methyl 4-nitroimidazole-5-carboxylate (California Biochemicals) with H₂ in the presence of 5% Pd on charcoal.

4-Aminoimidazole and 4-amino-5-imidazolecarboxylic acid. These compounds were prepared according to the method of RABINOWITZ³.

4-Formamido-5-imidazolecarboxamide. SHAW'S⁴ method was used for the preparation.

4-Ureido-5-imidazolecarboxylic acid. This was a gift from Dr. J. RABINOWITZ of the National Institute of Health, Bethesda, Maryland.

Glycocyamidine. An aqueous solution (pH 2) of glycocyamidine was boiled for 5 min. The product was isolated by paper chromatography in isopropanol-ammonium hydroxide-water (8:1:1). PCF was used as the spray reagent.

Taurocyamine. This was prepared from taurine and freshly prepared S-ethylthiourea hydrobromide in ammoniacal medium according to MORRISON *et al.*⁵. The final product was purified by crystallization from water and ethanol (1:10).

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF SYNTHESIZED STANDARDS

Compound	Color reactions to different spray reagents*										$R_F \times 100$ in the solvent							
	3500 A	2570 A	DSA	DNA	Ehr.	Ag ⁺	Nin.	DQC	DQC- base	Hg- DPC	An.	PCF	B.M.	1	2	3	4	5
Catechol monosulfate	—	—	Y	R	—	Gy	—	B	B	—	Bn	—	—	38	68	80	48	82
Catechol monophosphate	—	—	OY	R	—	Gy	—	Gy	Gy	—	Bn	—	—	46	5	53	54	39
4-Guanidinoimidazole	—	P	RP→V	OR	—	—	—	—	—	P	RBn	R	—	25	36	58	80	62
4-Guanidino-5-imidazole- carboxamide	—	—	R→P	—	—	—	—	—	—	—	—	R	—	42	38	55	81	—
Methyl 4-aminoimidazole- 5-carboxylate	b	P	OR	MV	—	—	—	vpBn	Y	P	—	—	R	58	63	73	81	79
4-Aminoimidazole	—	—	B	GyB	Y→OR	Gy	M	—	—	Gy	P	—	Gy	26	Dc**	58	70	Dc
4-Amino-5-imidazole- carboxylic acid	—	P	B	—	—	—	—	—	—	R	P	—	Gy	Dc	12	52	Dc	—
4-Formamido-5-imidazole- carboxamide	—	—	YGn	Y	Y	pGy	—	—	—	—	P	—	—	46	39	58	81	67
4-Ureido-5-imidazole- carboxylic acid	—	—	R	P	Y	—	—	—	—	R	RBn	—	—	23	25	53	60	53
Glycocyanidine	—	—	—	—	—	Gy	vpM	—	—	—	—	MP	—	33	38	56	78	54
Taurocyamine	—	—	—	—	—	—	—	—	—	P	—	R	—	14	24	55	45	52
Homotaurocyamine	—	—	—	—	—	—	—	—	—	P	—	R	—	11	22	54	44	40
4-Methoxy-3-hydroxy- mandelic acid	—	d	OR	P	—	Bn	—	GyB	B	vpB	O	—	—	67	39	58	57	58

* U.V.: b = weak blue; d = weakly defined, slightly darker than background; P = purple, darker than background. Colors: The following colors were as previously defined: B = blue; Bn = brown; Gy = gray; GyB = gray blue; M = mauve; MP = mauve purple; MV = mauve violet; O = orange; OR = orange red; OY = orange yellow; P = purple; R = red; RBn = red brown; RP = red purple; V = violet; Y = yellow; YGn = yellow green. Adjectives: p = pale; vp = very pale; — indicates no reaction.

** Dc = compound decomposed in the solvent.

Homotaurocyamine. This was prepared as in the method above; homotaurine being used as the starting material instead of taurine.

4-Methoxy-3-hydroxymandelic acid. The method of CORSON *et al.*⁶ for the synthesis of mandelic acid was modified as follows:

1.5 g (0.03 moles) of sodium cyanide, 5 ml of water, and 4.5 g (0.03 moles) of isovanillin were placed in a 50 ml beaker and stirred, while 8.5 ml of a saturated solution of sodium bisulfite and 9 g of ice were added over a period of 10 min. The layer of 4-methoxy-3-hydroxymandelonitrile which appeared was separated using a separatory funnel. The crude nitrile was at once transferred to an evaporating dish and 4.3 ml of concentrated hydrochloric acid was added. The hydrolysis was allowed to proceed in the cold for about 12 h, after which the mixture was heated on a steam bath to drive off the water and excess hydrochloric acid. The final product was dissolved in ethanol.

Chromatography

Each material was chromatographed unidimensionally in *n*-butanol-acetic acid-water (4:1:1) (solvent 1), isopropanol-ammonia-water (8:1:1) (solvent 2), methanol-*n*-butanol-benzene-water (2:1:1:1) (solvent 3), isobutyric acid-0.5 *N* NH₄OH (10:6) (solvent 4), and *n*-butanol-pyridine-water (1:1:1) (solvent 5). Each *R_F* value given in Table I is the average of at least three descending runs on Whatman No. 1 paper on different days. Color reactions under ultraviolet light and with the various spray reagents are also recorded in Table I and are those appearing after chromatograms were run in solvent no. 3.

Eleven spray reagents were used as described previously⁷. They were: diazotized sulfanilic acid (DSA), diazotized *p*-nitroaniline (DNA), Ehrlich's reagent (*p*-dimethylaminobenzaldehyde in ethanol and HCl) (Ehr.), ammoniacal silver nitrate (Ag⁺), Bratton-Marshall (nitrous acid, then coupling component) (B.M.), ninhydrin (Nin.), 2,6-dichloroquinonechlorimide (DQC), saturated borax sprayed over DQC (DQC base), mercury-diphenyl carbazone (Hg-DPC), anisidine (An.), and pentacyanoaquaferriate reagent (PCF).

The color description was correlated and recorded as described previously⁷. The chromatographic data obtained are summarized in the table.

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