The Preparation of Homogentisic Acid and of 2:5-Dihydroxyphenylethylamine

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In this paper is reported a convenient method for the preparation of homogentisic acid and a synthesis of 2:5-dihydroxyphenylethylamine. This amine is of interest as a potential intermediate in tyrosine metabolism in mammals (Neuberger, 1948) and also as a pressor substance. We have also included a few observations relating to the biological behaviour of these two compounds.

Preparation of homogentisic acid

This acid was synthesized soon after its discovery in alcaptonuric urine by Baumann & Fraenkel (1894) from 2:5-dimethoxybenzaldehyde by successive conversion to the alcohol, chloride and nitrile, followed by hydrolysis. The overall yield was very poor, due mainly to side reactions occurring in the conversion of the chloride to the nitrile. Schoepf & Winterhalder (1940), who studied systematically the formation of substituted phenylacetic acids by various methods, have found that a large excess of cyanide has to be used in such reactions if a good yield of the nitrile is to be obtained. We hoped that the chloride might be conveniently prepared by chloromethylation of 1:4-dimethoxybenzene; however, even under the most varied conditions, only the dichloromethyl compound could be obtained. Since, as will be shown later, homogentisic acid can be prepared from 2:5-dimethoxybenzaldehyde in good overall yield by another method, we did not explore the method of Baumann & Fraenkel (1894) any further.

Various other methods for the synthesis of homogentisic acid have been described, but the yields reported were in most cases very poor. Hahn & Stenner (1929) prepared 2:5-dibenzoyloxy-1-allylbenzene from hydroquinone, and claimed to have oxidized the allyl compound directly with very dilute ozone to benzoylhomogentisic acid in good yield. In our hands the method was not found to be very satisfactory. The benzoyl groups are very labile, and the material had, as Hahn & Stenner themselves found, to be rebenzoylated during the synthesis. Owing to the low concentration of ozone to be employed, the oxidation has to be carried out over many hours or even days, if moderately large amounts of material are used (cf. Schoepf & Winterhalder, 1940). Moreover, apart from homogentisic acid, other substances are produced in the oxidation. Hill & Short (1937) obtained good yields in the oxidation of 3-allyl-*o*-tolyl methyl ether with aqueous $KMnO_4$ containing the required amount of acetic acid. We have applied their method to the oxidation 1:4-dimethoxy-2-allylbenzene. This compound is readily obtained by Claisen rearrangement from 1-methoxy-4-allyloxybenzene (Mauthner, 1921) followed by methylation. However, oxidation of this compound with $KMnO_4$ gave, under the many conditions which were tried, a

mixture of substances, and the yield of the desired 2:5-dimethoxyphenylacetic acid was generally poor. Apart from 2:5-dimethoxyphenylbenzoic acid, a large amount of a neutral compound was obtained which was identified as 3-(2':5'-dimethoxyphenyl)-propane-1:2-diol. This glycol reacted with periodic acid to give formaldehyde and 2:5-dimethoxyphenylacetaldehyde which was not isolated, but oxidized directly to the corresponding acid. The diol was also prepared, albeit in poor yield, by oxidizing the allyl compound with performic acid (Swern, Billen & Scanlan, 1946; English & Gregory, 1947). Oxidation of *o*-hydroxyphenylacetic acid with persulphate gave homogentisic acid, but again the yield was poor.

Homogentisic acid was finally prepared in good yield by oxidation of 2:5-dimethoxyphenylpyruvic acid with H_2O_2 followed by hydrolysis. 2:5-Dimethoxybenzaldehyde was condensed with hippuric acid (Gulland & Virden, 1928; Neuberger, 1948) and the azlactone ring opened with alkali. Gulland & Virden (1928) then hydrolyzed the benzamidoacrylic acid and separated the keto and benzoic acids with SO_2. It was found more convenient to oxidize the mixture of the two acids directly, and to separate the benzoic and 2:5-dimethoxyphenylacetic acids by esterification and fractional distillation (see Snyder, Buck & Ide, 1943). Short hydrolysis with HBr yielded homogentisic acid in good overall yield. Two other methods have been published which might be equally suitable for the preparation of this acid. Schwenk & Bloch (1942) have prepared 2:5-dimethoxyphenylacetic acid by a modification of the Willgerodt reaction from the corresponding acetophenone and recently McElvain & Engelhardt (1944) have obtained the lactone of homogentisic acid by hydrolysis of 5-hydroxy-2-ethoxycoumarone which had been made by condensation of *p*-quinone with ketene acetal.

Synthesis of 2:5-dihydroxyphenylethylamine

The corresponding dimethoxy compound has been prepared by a Hofmann degradation of 3-(2':5'-dimethoxyphenyl) propionamide (Buck, 1932), and also by electrolytic reduction of 2:5-dimethoxy-ω-nitrostyrene (Sugasawa & Shigehara, 1941), the former method giving the better yield. In the present work the Curtius degradation was used. The propionic acid was made as described by Buck (1932), except that the cinnamic acid was reduced by H_2 under pressure, using Raney's nickel as catalyst. The propionic acid was esterified by the method of Baker, Querry, Safir & Bernstein (1947) and converted into the hydrazide and azide. Decomposition of the latter gave 2:5-dimethoxyphenylethylamine in excellent overall yield and on further treatment with HI, 2:5-dihydroxyphenylethylamine was obtained as the hydriodide which was converted with AgCl to the hydrochloride. The amine is easily oxidized by air,

even in acid solution. It is much more sensitive to O_2 than the corresponding catechol derivative which was prepared for comparison. The free base, which is not very soluble in water, has been obtained crystalline, but appears to be unstable even in the complete absence of O_2.

Biological observations

Homogentisic acid. It has been reported (Neuberger, Rimington & Wilson, 1947) that the blood level of homogentisic acid in the human alcaptonuric is vanishingly low at a time when the concentration in the urine is quite high. Various explanations for this finding were discussed. We have not been able to carry out further investigations on alcaptonurics, but had to confine ourselves to normal subjects. Ingestion of homogentisic acid (H.A.) in quantities up to 5 g. did not produce alcaptonuria, nor a measurable increase of reducing substances in the blood. Intravenous injection, however, of quantities of 0·3–1·0 g. produced a marked alcaptonuria which lasted, at the most, for only 40 min. The total amount of H.A. excreted varied between 28 and 64 % of the administered dose, the rest being presumably metabolized. The blood level of H.A. was, in all experiments, well below 15 mg./100 ml., and in some cases of the order of 2–5 mg./100 ml. The accuracy of the estimation of H.A. in serum or plasma is low with present methods (Neuberger et al. 1947), especially with the very small quantities involved, and no quantitative significance can be attached to the plasma figures. Nevertheless, it is possible to arrive at certain conclusions. In several experiments, e.g. 0·3 g. H.A. was injected, and on the average a third of the injected dose was recovered in the urine. After H.A. had completely mixed with the blood, the concentration in the whole blood could not have been greater than 6 mg./100 ml., if the blood volume is assumed to be about 5 l. If allowance is made for oxidation in the body and diffusion into the intercellular space, the concentration was probably appreciably lower. The values found, 1·5–3·8 mg./100 ml., are therefore quite reasonable. It thus appears that, even in normal man, the kidney threshold for H.A. is well below 4 mg./100 ml. In normals alcaptonuria cannot be produced by feeding large quantities of tyrosine by mouth, and it must therefore be assumed that H.A., if it is an intermediate, never appears in the blood in appreciable amounts, and is presumably rapidly oxidized in the organ in which it is first formed. The alcaptonuric has probably the same low kidney threshold as the normal, but he excretes H.A. in high concentrations in the urine, though the acid is either absent from the blood or present in minute amounts only. A reasonable explanation of these findings is that H.A. is formed and normally further metabolized in the kidney. No accumulation of H.A. occurs since its destruction is faster than its formation. In experi-mental alcaptonuria this destruction may be slowed down somewhat, some H.A. accumulates and is excreted owing to its low threshold. In human alcaptonuria, the inhibition of further oxidation may be more complete, and consequently a much larger proportion of H.A. is eliminated in the urine.

2:5-Dihydroxyphenylethylamine. This amine is a pressor substance of somewhat weaker potency than 3:4-dihydroxyphenylethylamine. A more detailed study of the pharmacological properties of the new amine has been undertaken in the Pharmacological Department, University of Oxford, and will be published elsewhere. The 2:5-dihydroxyphenylethylamine is toxic, if given by vein, to the mouse; a dose of 125 mg./kg. body weight was found to be lethal. But given orally, the toxicity is low, at least in the rat. Thus 200–300 mg./day were tolerated quite well by adult animals (300 g.). The amine appears to be readily metabolized since the administration of such large doses did not produce any reducing material in the urine. Moreover, no hydroquinone derivatives could be detected after treatment of the urine with acid. Thus four derivatives of hydroquinone, viz. 2:5-dihydroxyphenylalanine (Neuberger, 1948), the corresponding ethylamine and the acetic and pyruvic acids are all readily metabolized by the rat. This is in marked contrast to the behaviour of similar derivatives of catechol. 3:4-Dihydroxyphenylalanine is only incompletely metabolized by man (Guggenheim, 1913), the rabbit (Fromherz & Hermanns, 1914) and the rat (Holtz & Credner, 1944), and the same applies to 3:4-dihydroxyphenylethylamine, as far as the rat is concerned. In the present work it was found that 3:4-dihydroxyphenylacetic acid is largely excreted unchanged by the rat, even if only relatively small doses are given. Homogentisic acid, on the other hand, is readily metabolized. It thus appears that the mammalian organism is much better adapted to the oxidation of hydroquinone than of catechol derivatives, and this supports the conclusion that the former are normal intermediates in metabolism.

EXPERIMENTAL

Preparation of compounds

1:4-Dimethoxy-2:5-di-(chloromethyl)-benzene. A mixture of 1:4-dimethoxybenzene (69 g.), 40 % formaldehyde solution (37·5 ml.) and 36 % (w/v) HCl (350 ml.) was stirred for 6 hr. at 55°, whilst a slow stream of HCl was passed through the solution. The product was taken up in ethyl acetate, washed thoroughly with water and the ethyl acetate solution dried and concentrated. Distillation of the remaining oil *in vacuo* (0·1 mm.) gave unchanged 1:4-dimethoxybenzene (32 g.) and the dichloromethyl ether (34 g.). The m.p. of the latter was 165–166° (after recrystallization from benzene). This substance has previously been prepared by I.G. Farbenindustrie (British Patent no. 347,892) and is

stated to have m.p. 165°. Variation of the conditions such as reducing the reaction time and temperature did not appreciably affect the result.

Oxidation of 1:4-*dimethoxy*-2-*allylbenzene*

Oxidation with permanganate in acetone. To a solution of 1:4-dimethoxy-2-allylbenzene (17·4 g.) in acetone (100 ml.) was added over a period of 2 hr. KMnO₄ (52 g.) in 70% (v/v) acetone (800 ml.). The solution was stirred and the temperature kept at 25–30°, the MnO_2 which was filtered off was extracted three times with boiling water (150 ml. each), and the combined solutions treated with SO_2 till colourless. The solution was then made alkaline and extracted with ether; this extract is the 'neutral' fraction. The aqueous solution was then made acid to congo red, concentrated *in vacuo* to low vol. and again extracted exhaustively with ether. The 'acidic' ethereal solution was concentrated to dryness, and the residue taken up in hot water, treated with charcoal and cooled. The crystalline material was collected and recrystallized from water. It had m.p. 125° and an acid equiv. of 194; calc. for $C_{10}H_{12}O_4$ 196. Wolkow & Baumann (1891) give m.p. of 2:5-dimethoxyphenylacetic acid 124·5°. The mother liquor gave a further crop of crystalline material, fractional crystallization of which gave some more of the acetic acid and also 2:5-dimethoxyphenylbenzoic acid of m.p. 76°; the latter on hydrolysis gave gentisic acid. The yield of 2:5-dimethoxyphenylacetic acid varied between 15 and 18%. Lowering of the temperature, on reduction of the amount of KMnO₄ used, did not appreciably increase the yield.

3-(2′:5′-*Dimethoxyphenyl*)-*propane*-1:2-*diol*. The 'neutral' fraction of the above oxidation was concentrated to dryness, and the crystalline residue recrystallized from light petroleum (b.p. 120–130°). It had m.p. 77·5°. (Found: C, 62·2; H, 7·7. $C_{11}H_{16}O_4$ requires: C, 62·2; H, 7·55%.) Oxidation with periodic acid showed the substance to be a 1:2-glycol; 0·106 g. of the diol was dissolved in a little warm water and added to 0·42M-periodic acid (2 ml.). The solution which became cloudy almost at once, due to the formation of the phenylacetaldehyde, was left for 1·5 hr. It was then extracted with ether, and the excess periodic acid titrated with 0·1 N-arsenite, 1·17 ml. periodic acid had been used up; $C_{11}H_{16}O_4$ requires 1·19 ml. In another experiment the formation of formaldehyde was demonstrated by the isolation of the dimedone derivative.

Oxidation with performic acid. A mixture of 30·5% (w/v) H₂O₂ (35 ml.) and formic acid (50 ml.) was allowed to stand at 18° for 0·5 hr. It was then added in three equal portions over 15 min. to a stirred mixture of 1:4-dimethoxy-2-allylbenzene (53·4 g.) and formic acid (90 ml.). After a lag period of 5–10 min. the reaction started; the temperature was kept at 45–55° by cooling occasionally with ice water. After the exothermic reaction had subsided, the now homogeneous solution was kept at 50–60° for another 1·5 hr. The solution was concentrated under reduced pressure, and the residue refluxed with a slight excess of N-NaOH in ethanol. The solution was neutralized, shaken with water and ether, and the ethereal solution dried. Removal of the ether gave a residue which was recrystallized three times from light petroleum (b.p. 120–130°). The diol was identified by its m.p. and titration with periodic acid. Yield was 20%. Swern *et al.* (1946) have obtained much better yields with purely aliphatic olefins.

Oxidation with permanganate in water containing acetic acid

1:4-Dimethoxy-2-allylbenzene (20 g.) was suspended in water (2 l.) containing acetic acid (46 ml.), and the mixture stirred into an emulsion. KMnO₄ (57 g.) dissolved in water (1·1 l.) was then added over 7 hr., whilst the temperature was kept at −1 to 0°. On working up the solution, as described by Hill & Short (1937), a small amount of 2:5-dimethoxyphenylacetic acid was obtained in addition to some diol.

Preparation of homogentisic acid from 2-*phenyl*-4-(2′:5′-*dimethoxybenzylidene*)-*oxazolone*

2:5-*Dimethoxyphenylacetic acid.* The azlactone was converted to 2:5-dimethoxyphenylpyruvic acid as described by Gulland & Virden (1928). The crude keto acid (22·6 g.) was oxidized with 8·4 M-H₂O₂ (12·5 ml.) and 10 N-NaOH (12·75 ml.) as described by these authors. At the end of the oxidation the alkaline solution was extracted with ether and then acidified with 36% (w/v) HCl (55 ml.). The solution was then again extracted with ether, and the ethereal solution dried and concentrated. The solution, which was obtained on adding ethanol (150 ml.) and conc. H₂SO₄ (2·75 ml.) to the remaining oil, was refluxed for 7 hr. with exclusion of moisture. Most of the ethanol was then removed by distillation under reduced pressure, water (75 ml.) was added, and the two layers separated. The aqueous solution was extracted with ethyl acetate, and the combined oil and ethyl acetate extracts washed with NaHCO₃. After drying and removal of the solvent, the oil was distilled at 20 mm. The first fraction (b.p. 102–104°) consisted of ethyl benzoate, whilst the second fraction which amounted to 16 g. distilled at 170–175°. The latter material was saponified by boiling with 2·5 N-NaOH for 0·5 hr., extracted with ether and acidified. To the white precipitate was added enough water to dissolve most of the material on boiling, a small amount of oil was discarded, and the solution treated with charcoal. On filtration and cooling crystalline material was obtained which was recrystallized twice from water. M.p. was 125°. Yield after recrystallization was 70%.

Homogentisic acid. The dimethoxy acid (7·8 g.) was hydrolyzed by boiling with 120 ml. HBr (sp.gr. 1·48) for 5 hr. The solution was concentrated *in vacuo* under N₂, and the residue taken up in hot water. This solution was again concentrated until crystals appeared. This solution containing some crystals was extracted three times with 10 vol. of ether. The ethereal solution, after drying, was concentrated until crystallization began and chloroform was added. The crystalline material was filtered off and identified as homogentisic acid. It had m.p. 147–148°, gave the typical reaction with FeCl₃ and reduced Ag⁺ in acid solution. Yield was 80% of the theoretical.

Preparation of 2:5-*dihydroxyphenylethylamine*

3-(2′:5′-*Dimethoxyphenyl*)-*propionic acid*. 3-(2′:5′-Dihydroxyphenyl)-acrylic acid (77 g.) was dissolved in 0·5 N-NaOH (650 ml.) and hydrogenated with Raney nickel (10 g. suspension) and hydrogen at 100 atm. and at room temperature. The theoretical quantity of hydrogen was taken up after 1 hr. The catalyst was filtered off and the solution made acid to congo red. After cooling the product

was filtered off; it amounted to 74·5 g. After recrystallization from light petroleum (b.p. 120–130°) it had m.p. 66–67°. The material thus obtained was apparently mainly in the form of a hydrate, in spite of having been dried over P_2O_5 at 0·1 mm. (Found: C, 59·0; H, 6·95. Calc. for $C_{11}H_{14}O_4$: C, 62·8; H, 6·6; calc. for $C_{11}H_{14}O_4H_2O$: C, 58·0; H, 7·02%.) Buck (1932) gives for the anhydrous acid m.p. 101°. The hydrated material was dried at 110° and at 0·1 mm. over P_2O_5 for 3 hr. The oil was then crystallized from light petroleum (b.p. 120–130°). The crystalline material now had m.p. 100° in agreement with Buck.

Ethyl 3-(2′:5′-dimethoxyphenyl) propionate. A mixture of 70 g. of the above acid, ethanol (100 ml.), benzene (150 ml.) and conc. H_2SO_4 (10 ml.) was refluxed for 10 hr. in a Soxhlet apparatus containing anhydrous $MgSO_4$ (50 g.) in the thimble. The cooled mixture was washed with water and the solvents removed. The ester distilled at 152–154° (1·5 mm.). Yield was 90–95%.

3-(2′:5′-Dimethoxyphenyl) propionic hydrazide. The above ester (38·4 g.) was refluxed for 6 hr. with 90% hydrazine hydrate (22 ml.) and addition of sufficient amyl alcohol to produce a homogeneous solution. The mixture was cooled, diluted with ether and the hydrazide extracted with 2N-HCl. On neutralization with NaOH the hydrazide precipitated in crystalline form. After cooling it was filtered off and dried. On recrystallization from benzene or water m.p. of the *hydrazide* was 95–96°. (Found: C, 58·9; H, 7·1; N 12·6. $C_{11}H_{16}O_3N_2$ requires: C, 58·9; H, 7·1; N, 12·5%.)

2:5-Dimethoxyphenylethylamine. To a solution of the hydrazide (34 g.) in 5N-HCl (153 ml.), stirred at 0°, was added rapidly a solution of $NaNO_2$ (10·5 g.) in water (20 ml.). Ice was added to keep the temperature below 10°. The azide, which separated as a pale-yellow oil, was extracted with cold benzene, and the benzene solution washed with ice-cold saturated NaCl. The solution was then dried at 0° over Na_2SO_4 and then over $CaCl_2$ and filtered. It was then warmed cautiously until evolution of N_2 began. After the reaction had abated, the solution was refluxed with exclusion of moisture for 20 min. Most of the solvent was distilled off, the residue was cooled and treated with cold HCl (200 ml. saturated at 0°). On warming, CO_2 was evolved. When the reaction appeared to be complete, most of the HCl was distilled off, leaving a crystalline residue of the hydrochloride. This had m.p. 142–143° after recrystallization from ethanol. The bulk of the hydrochloride was decomposed with excess NaOH, and the amine extracted with benzene. The benzene extract was dried and solvent removed. The amine distilled at 100° (0·5 mm.). Yield was 80%.

2:5-Dihydroxyphenylethylamine hydrochloride. All operations from now on were carried out, as far as possible, in an atmosphere of N_2. The above amine was hydrolyzed by refluxing for 20 min. with 5 vol. of HI (sp.gr. 1·7) which had been freshly distilled over red phosphorus. Excess HI was removed under reduced pressure leaving a pale-yellow oil which could be crystallized from acetone-ether. It had m.p. 173°. The bulk of the hydriodide was converted to the hydrochloride as follows: amine hydriodide (5·0 g.) was dissolved in water (15 ml.) containing a trace of SO_2; the solution was shaken with freshly prepared AgCl (3·0 g.) with addition of 5N-HCl (0·5 ml.). The mixture of AgCl and AgI was filtered off, washed with water, and the filtrate and washings concentrated to dryness. The oil which remained was dissolved in a small amount of ethanol and crystallized

by addition of dry ether. The hydrochloride was difficult to crystallize, owing to its great solubility and the ease with which it is oxidized, and it was found advisable to work with small amounts (2–3 g.). After several recrystallizations from ethanol-ether the *amine hydrochloride* had m.p. 169–170°. (Found: C, 50·8; H, 6·3; N, 7·1; Cl, 18·6. $C_8H_{12}O_2NCl$ requires: C, 50·6; H, 6·3; N, 7·4; Cl, 18·8%.) The amine formed a crystalline diliturate; however, this salt decomposed during recrystallization. The free amine which was prepared from the hydrochloride by addition of 1 equiv. of NaOH, crystallized well from water and had m.p. 128–130°. But attempts to recrystallize the base, in an atmosphere of N_2, were unsuccessful owing to its instability. On keeping, the free base, sealed under nitrogen, decomposed within a few days to a black tar.

METABOLIC EXPERIMENTS

Methods

Feeding experiments and estimation of homogentisic acid in the urine were carried out as described by Neuberger (1947, 1948).

In experiments on man, H.A. was dissolved in pyrogen-free water (twice glass-distilled), and sufficient $NaHCO_3$ was added to neutralize about 90% of the acid. This solution was then passed through a Seitz filter, and the concentration of H.A. estimated iodometrically immediately before use. The final concentration was between 3 and 4%. The solution was then quickly injected into the antecubital vein; samples of 5 ml. blood were withdrawn before the injection and at intervals of 5 min., after the injection. H.A. was estimated by the micro-iodometric and by the Folin method (Neuberger *et al.* 1947), and H.A. added to plasma before removal of the protein was used as standard.

Results

Toxicity of 2:5-dihydroxyphenylethylamine

The mice used for these experiments weighed between 20 and 25 g. 1 mg. of the amine hydrochloride (1% solution in water) given by vein to three mice had no effect; 2·5 mg. produced immediate death in two out of three animals; 5 mg. produced immediate death in three out of three mice. The surviving animals were observed for 3 days and appeared normal.

Feeding experiments on rats

Homogentisic acid. This was given by mouth in doses of 50–500 mg./day to ten rats kept on normal diets; there were no reducing substances in the urine, either before or after hydrolysis with an equal amount of 1·5N-HCl at 100° for 1 hr.

2:5-Dihydroxyphenylethylamine hydrochloride. This was given by mouth to seven rats in daily doses of 50–300 mg. There were no toxic effects, and no reducing substances could be demonstrated in the urine.

3:4-*Dihydroxyphenylacetic acid*. The acid was given to five rats in daily doses of 25–100 mg. In all experiments the urine collected over the 24 hr. following the administration reduced Ag^+ in alkaline solution, and gave a strong colour reaction with $FeCl_3$. The reducing material was not isolated, but appears to have been an acid. It could be extracted into ether from an acid, but not from a neutral, aqueous solution and was most probably unchanged material. A rough semi-quantitative estimation with the aid of the $FeCl_3$ reaction indicated that about 75 mg. of the 100 mg. given were excreted.

Experiments on man

These experiments were done on two healthy males, weighing 63 and 69 kg. respectively.

Ingestion by mouth. Amounts of H.A. of 1–5 g. dissolved in 10 vol. of water were taken by mouth with and without an equivalent amount of $NaHCO_3$. Urine was collected for 48 hr. Results were completely negative.

Injection by vein. Altogether, seven experiments were carried out, four with 0·3 g. H.A. and three with 1·0 g. The results with the two subjects were similar. With the smaller dose the excretion of H.A. in the urine began within 5 min. and lasted for 15–25 min. and most of the acid was eliminated in the first 10 min. The plasma level of H.A., 5 min. after the injection, varied between 2·5 and 3·8 mg./100 ml. to return to the initial value after about 15 min. With the bigger dose, the urinary excretion of H.A. persisted for 28–40 min., the bulk again being eliminated in the first 10–15 min. The plasma level after 5 min. varied between 8·0 and 10·5 mg./100 ml. and returned to normal within 20–25 min. The total amount excreted was 28–35 % of the injected dose with 0·3 g. H.A. and 48–64 % with 1·0 g. H.A.

SUMMARY

1. The preparation of homogentisic acid by various methods has been studied. The most convenient synthesis found consisted of oxidation of 2:5-dimethoxyphenylpyruvic acid by hydrogen peroxide, followed by demethylation.

2. 2:5-Dihydroxyphenylethylamine has been prepared by a Curtius degradation of the corresponding hydrazide.

3. This amine was found to be readily metabolized by the rat, and thus resembles other derivatives of hydroquinone. 3:4-Dihydroxyphenylacetic acid, on the other hand, was excreted largely unchanged as indicated by the reducing action of the urine.

4. Homogentisic acid, given by vein but not by mouth, to man, produced an alcaptonuria of short duration. It is concluded that the renal threshold for this acid is very low, even in the normal.

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