followed by the slow addition of 0.22 mole freshly distilled acetyl chloride. After 4 h, the precipitated triethylammonium chloride was removed by filtration and the filtrate evaporated to give 71 % of crude N,N'-diacetyl-L-cystine dimethyl ester. 0.05 mole of this product was dissolved in a mixture of 250 ml distilled water and 60 ml conc. HCl and to this solution Br_2 was added, slowly and with stirring, until a slight excess was present. Approximately 0.25 mole was required. The resulting solution was concentrated *in vacuo* to give 75 % of crude N-acetyl-L-cysteic acid carboxymethyl ester, m.p. 186° with decomp. A solution of 0.04 mole of the crude ester in 1 l dry methanol was saturated with anhydrous ammonia and the reaction mixture maintained at 25° in a sealed vessel for 5 days. The reaction mixture was then evaporated to dryness and the residue recrystallized from anhydrous ethanol to give the ammonium salt of N-acetyl-L-cysteic acid carboxamide, m.p. 201–202°, $[\alpha]_D^{25} = -9.3°$ (in water). Yield from L-cystine, 18 %.

Anal. Calcd. for $C_5H_{13}O_5N_3S$ (227): C, 26.4; H, 5.7; N, 18.5; S, 14.1. Found: C, 26.4; H, 5.8; N, 18.5; S, 14.0.

The conditions employed in examining the action of α-chymotrypsin on the ammonium salt of N-acetyl-L-cysteic acid carboxamide are summarized in Table I. The α-chymotrypsin was an Armour preparation, lot No. 00592.

The procedure employed for observing the effect of added ammonium N-acetyl-L-cysteate carboxamide upon the α-chymotrypsin-catalyzed hydrolysis of α-N-acetyl-L-tyrosinehydrazide (Table II) was identical to that described previously[4] and again the enzyme preparation was crystalline α-chymotrypsin, Armour lot No. 00592.

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[1] F. SANGER AND E. O. P. THOMPSON, *Biochem. J.*, 53 (1953) 366.
[2] H. NEURATH AND G. W. SCHWERT, *Chem. Revs.*, 46 (1950) 69.
[3] R. J. FOSTER AND C. NIEMANN, *J. Am. Chem. Soc.*, 77 (1955) 1886.
[4] H. T. HUANG AND C. NIEMANN, *ibid.*, 73 (1951) 1541.
[5] R. LUTWACK, H. F. MOWER AND C. NIEMANN, *ibid.*, 79 (1957) 2179.
[6] D. F. ELLIOTT, *Biochem. J.*, 50 (1952) 542.

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3-Methoxy-4-hydroxy-D-mandelic acid, a urinary metabolite of norepinephrine*

One of the phenolic acids in human urine, compound 10[1], appears to be of endogenous origin, since its excretion is not affected by dietary changes. Many of the qualitative reactions of this substance were observed to be similar to those of some compounds containing the 3-methoxy-4-hydroxyphenyl group. This fact, along with the recent observation that homoprotocatechuic acid undergoes biological methylation to homovanillic acid[2], and the solubility characteristics of the unknown substance as indicated by its chromatographic behavior were suggestive that it might be 3-methoxy-4-hydroxymandelic acid (I). (I) might be expected to be formed by the action of amine oxidase upon norepinephrine or epinephrine[3], followed by methylation of the resulting 3,4-dihydroxymandelic acid. The following results indicate that (I) is, indeed, an important urinary metabolite of norepinephrine: (1) The parenteral administration of norepinephrine leads to an increased amount of (I) in the urine**; (2) orally ingested 3,4-dihydroxy-DL-mandelic acid gives rise to an increased excretion of (I); and (3) three patients with pheochromocytomas excreted greatly increased amounts of (I) preoperatively and normal amounts postoperatively***.

Authentic DL-(I) was prepared by the method of GARDNER AND HIBBERT[4]; m.p. 129–130° dec.§. L- and D-(I) were prepared by fractional crystallization of the cinchonine salt of DL-(I). The

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§ All melting points were made in open capillary tubes and are uncorrected.

first crop of less soluble salt was enriched in D-(I) and the mother liquor was enriched in L-(I). Impure L- and D-(I) were regenerated from their crude salts and recrystallized (acetonitrile) to constant rotation. L-(I), m.p. 151° dec., $[\alpha]_D^{25}$, +133 (1% in water); D-(I), m.p. 152° dec., $[\alpha]_D^{23}$, −133° (1% in water). The cinchonine salt of D-(I) was recrystallized (abs. ethanol) to constant rotation; $[\alpha]_D^{24}$, +89° (1% in EtOH), m.p. 203–204° dec. D-(I) was regenerated from this salt; $[\alpha]_D^{23}$, −131° (1% in water), m.p. 152° dec. L-(I), m.p. 152° dec., $[\alpha]_D^{22}$, +128 (0.7% in water) was also prepared by enzymic hydrolysis of DL-(I)-amide with purified leucine aminopeptidase. The enzymic resolution served for assignment of configuration since leucine aminopeptidase shows an absolute specificity for amides having the L-configuration[5]; the aminopeptidase hydrolyzes α-hydroxy acid amides as well as α-amino acid amides[6] *.

D-(I) was isolated from the urine of a patient with a pheochromocytoma. Urine (14 l) containing an estimated 135 mg of (I) was extracted with ethyl acetate and the organic acids were separated in the manner described previously[1]. For initial purification, the extract of organic acids was subjected to a modified countercurrent extraction procedure[7], a portion of the resulting purified concentrate (containing about 60 mg (I)) was applied to Whatman 3 MM filter paper (about 0.5 mg (I) per spot) and subjected to ascending chromatography with *iso*propyl alcohol–aqu. NH_3–water (8:1:1). The area containing most of the (I) was eluted and 30 mg of crude D-(I) was obtained by digesting the concentrated eluate with acetonitrile. The crude material was recrystallized from 0.4 ml of acetonitrile to yield 19 mg of pure D-(I); m.p. 152° dec. (not depressed by admixture with authentic D-(I)); $[\alpha]_D^{22}$, −138 (0.7% in water). The crystal form, solubility, and chromatographic properties of the isolated material were the same as those of authentic D-(I).

3,4-Dihydroxy-DL-mandelic acid was prepared from protocatechualdehyde via the cyanohydrin, imino ester hydrochloride, and ethyl ester; m.p. 138° dec. Analysis: Calculated for $C_8H_8O_5$: C, 52.18, H, 4.38; Found: C, 51.46, H, 4.35. This acid had been prepared previously in a very impure state by BARGER AND EWINS[8].

(I) in urine may be estimated by two dimensional paper chromatography of an extract of the organic acids prepared as described previously[1]. For chromatography, aliquots of extract containing about 1 γ of (I) are applied to paper, along with suitably placed standard quantities (0.5, 1.0, 1.5, and 2.0 γ) of authentic (I). For the first system, *iso*propyl alcohol–aqu. ammonia–H_2O (8:0.2:1.8), R_F, 0.40, and for the second system, benzene–propionic acid–H_2O (100:70:5), R_F, 0.16, give good results. The chromatograms are sprayed with diazotized sulfanilic acid[9] and the amount of (I) is estimated by comparing it with the standard spots. The extraction procedure recovers 90–95% of added (I), and the accuracy of the estimation is about ±15%. Normal adults excrete (I) at the level of 1.5 to 3.0 γ/mg creatinine. The three patients with pheochromocytoma excreted 90, 23, and 12 γ (I)/mg creatinine preoperatively and 2.7, 4.4, and 1.5 postoperatively, respectively.

It is likely that (I) is also a metabolite of epinephrine, since both amines are substrates for amine oxidase[3] and the distribution of radioactive compounds in the urine of rats has been shown to be similar after the administration of ^{14}C-epinephrine and norepinephrine[10]. Under normal conditions, however, the daily excretion of norepinephrine is about five times that of epinephrine[11]. Estimation of (I) in urine will probably be of value for the detection of pheochromocytomas, and in physiological studies of the production of norepinephrine in various other conditions.

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1 M. D. ARMSTRONG, K. N. F. SHAW AND P. E. WALL, *J. Biol. Chem.*, 218 (1956) 293.
2 K. N. F. SHAW, A. MCMILLAN AND M. D. ARMSTRONG, *J. Biol. Chem.*, 226 (1957) 255.
3 U. S. VON EULER, *Noradrenaline*, C.C. Thomas, Springfield, 1956, pp. 41–53.
4 J. A. F. GARDNER AND H. HIBBERT, *J. Am. Chem. Soc.*, 66 (1944) 607.
5 E. L. SMITH AND D. H. SPACKMAN, *J. Biol. Chem.*, 212 (1955) 271.
6 R. L. HILL AND E. L. SMITH, *J. Biol. Chem.*, 224 (1957) 209.
7 M. D. ARMSTRONG AND K. N. F. SHAW, *J. Biol. Chem.*, 225 (1957) 269.
8 G. BARGER AND A. J. EWINS, *J. Chem. Soc.*, 95 (1909) 552.
9 H. K. BERRY, H. E. SUTTON, L. CAIN AND J. S. BERRY, *Univ. Texas Publ.*, No. 5109 (1951) 22.
10 R. W. SCHAYER, R. L. SMILEY, K. J. DAVIS AND Y. KOBAYASHI, *Am. J. Physiol.*, 182 (1955) 285.
11 U. S. VON EULER, S. HELLNER-BJÖRKMAN AND I. ORWÉN, *Acta Physiol. Scand.*, 33, Suppl. 118 (1955) 10.

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