Isolation of *iso*Xanthopterin from Human Urine

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The pterins xanthopterin (2-amino-4:6-dihydroxypteridine) (Koschara, 1936) and biopterin (2-amino-4-hydroxy-6-[1:2-dihydroxypropyl-(L-*erythro*)]-pteridine) (Patterson, Saltza & Stokstad, 1956) have been isolated from human urine. It has been suggested that the pterins 2-amino-4-hydroxypteridine-6-carboxylic acid, 2-amino-4-hydroxypteridine and *iso*xanthopterin (2-amino-4:7-dihydroxypteridine) are products of catabolism of pteroyl-L-glutamic acid (Blair & Graham, 1955). It has also been shown that pteroyl-L-glutamic acid is converted by methylene blue and xanthine oxidase into 2-amino-4-hydroxypteridine-6-carboxylic acid (Blair, 1957). It seemed desirable, therefore, to examine human urine further.

When freshly voided adult male urine was analysed, as described below, a small quantity of a purple fluorescent substance could be isolated. On further purification this substance was shown to be *iso*xanthopterin, by its similar behaviour to an authentic sample in six solvent systems.

No 2-amino-4-hydroxypteridine-6-carboxylic acid could be isolated from freshly voided human urine. If, however, samples were allowed to stand, or if the analysis was unduly protracted, a bright-blue fluorescent substance could be isolated. This was not separable from 2-amino-4-hydroxypteridine-6-carboxylic acid in five different solvent systems. It was assumed that this acid was derived from the decomposition of biopterin in the urine.

The method used was not suitable for the isolation of 2-amino-4-hydroxypteridine.

EXPERIMENTAL

Conc. HCl (25 ml.) was added to adult male urine (250 ml.) which had been freshly voided into a flask containing a trace of phenol. The urine was then extracted with liquid phenol (50 ml.; Cranmer, 1948) and the phenol layer shaken up with water (25 ml.) and ether (100 ml.). The aqueous layer was washed twice with ether (100 ml. portions) and then evaporated to dryness under reduced pressure.

The residue was dissolved in water (5 ml.) and a few drops of aq. NH_3 were added until the solution was alkaline. The solution was filtered and evaporated to dryness under reduced pressure and the residue dissolved in aq. 0·5N-NH_3 (0·5 ml.).

The dark-brown, syrupy liquid was spotted on Whatman no. 3MM paper so that it filled a rectangle 3 in. × 1 in. The paper was then developed (descending, overnight, in darkness) with propanol–5% acetic acid (2:1, v/v), air-dried and viewed in 365 mμ light.

Three fluorescent areas could be seen: nearest the origin, a well-defined purple–blue band (1); next, a bright-yellow band (2); finally, a smaller yellow band (3). After the third yellow band the chromatogram was badly streaked. The first band was cut out and eluted with aq. 0·5N-NH_3. Further purification by chromatography, with no. 3MM paper and *tert.*-butyl alcohol–pyridine–water (50:15:35, by vol.), resolved this into three subsidiary bands. The eluate of the middle band (R_F 0·3–0·4) was chromatographed on no. 1 Whatman paper with propanol–aq. 1% NH_3 (2:1, v/v). Finally the eluate from this last chromatogram was evaporated to dryness under reduced pressure and the residue dissolved in aq. 0·5N-NH_3 (0·05 ml.). This solution was used for identification.

The purple fluorescent substance showed identical chromatographic behaviour on Whatman no. 1 paper with that of an authentic sample of *iso*xanthopterin, in butanol–acetic acid–water (4:1:5, by vol.; Good & Johnson, 1949), propanol–5% acetic acid (2:1, v/v; Forrest & Mitchell, 1954*a*), propanol–1% aq. NH_3 (2:1, v/v; Hadorn & Mitchell, 1951), propanol–N-HCl (2:1, v/v; Forrest & Mitchell, 1954*b*), and *tert.*-butyl alcohol–pyridine–water (50:15:35, by vol.; Forrest & Todd, 1950).

With *tert.*-butyl alcohol–pyridine–water (60:15:25, by vol.; Forrest & Todd, 1950) a difference of 0·06 was found in the R_F values of the unknown and *iso*xanthopterin. A mixture of the unknown and *iso*xanthopterin, however, did not separate into two spots and had a similar R_F to the unknown. This difference in R_F values of the unknown and *iso*xanthopterin is therefore due to the presence of impurities.

An additional amount of *iso*xanthopterin could be obtained by eluting band (2) and purifying it as before. In one experiment band (1) did not appear and *iso*xanthopterin was isolated from band (2).

For each identification 5μl. of solution was used and the dried papers were viewed in 365 mμ light. Papers were also viewed in 254 mμ light. This was less satisfactory as these pterins do not fluoresce so brilliantly as in 365 mμ light.

If the samples of urine were allowed to stand, or if the preliminary stages of the analysis were protracted, an additional bright blue band was found between band (1) and the origin. This band was eluted with aq. 0·5N-NH_3. It was not separable from authentic 2-amino-4-hydroxypteridine-6-carboxylic acid in five solvent systems. It was probably, therefore, 2-amino-4-hydroxypteridine-6-carboxylic acid formed from the decomposition of biopterin.

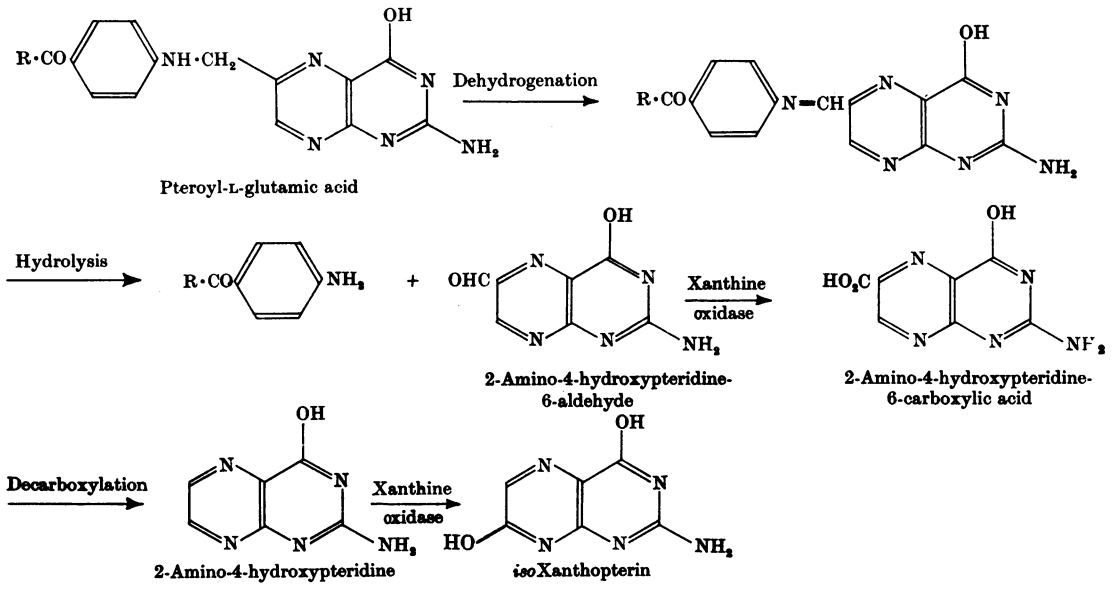

Fig. 1. Catabolism of pteroyl-L-glutamic acid. R represents $HO_2C{\cdot}[CH_2]_2{\cdot}CH(CO_2H){\cdot}NH{\cdot}$.

DISCUSSION

It has been suggested that pteroyl-L-glutamic acid is catabolized in the body by some yet unknown route (Williams, Eakin, Beerstecher & Shive, 1950). It is now suggested that the catabolism of pteroyl-L-glutamic acid proceeds as in Fig. 1.

Evidence for the conversion of pteroyl-L-glutamic acid into 2-amino-4-hydroxypteridine-6-carboxylic acid by processes of dehydrogenation, hydrolysis and enzymic oxidation has been described in a previous paper (Blair, 1957).

The pterins 2-amino-4-hydroxypteridine-6-carboxylic acid, 2-amino-4-hydroxypteridine and *iso*xanthopterin are widely distributed in Nature (Forrest, Van Baalen & Myers, 1957; Viscontini, Kuhn & Egelhaaf, 1956; Forrest & Mitchell, 1955; Viscontini, Schmid & Hadorn, 1955; Viscontini, Loeser, Karrer & Hadorn, 1955; Nawa & Taira, 1954; Hama, 1953; Busnel & Drilhon, 1949; for earlier references see Blair & Graham, 1955).

2-Amino-4-hydroxypteridine-6-carboxylic acid can be decarboxylated by heat (Forrest & Mitchell, 1954*b*) or by ultraviolet light (Lowry, Bessey & Crawford, 1949*a*; Viscontini, Loeser & Egelhaaf, 1956) to 2-amino-4-hydroxypteridine; and this can be oxidized by xanthine oxidase to *iso*xanthopterin (Lowry, Bessey & Crawford, 1949*b*).

It is therefore reasonable to suppose that these three pterins are intermediate in the biological degradation of pteroyl-L-glutamic acid. The isolation of *iso*xanthopterin from human urine supports this scheme. Further work is in progress.

SUMMARY

1. *iso*Xanthopterin has been isolated from human urine.

2. 2-Amino-4-hydroxypteridine-6-carboxylic acid could not be isolated from freshly voided human urine.

3. A scheme for the catabolism of pteroyl-L-glutamic acid is suggested.

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Studies on Sulphatases

19. THE PURIFICATION AND PROPERTIES OF ARYLSULPHATASE B OF HUMAN LIVER*

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Evidence is accumulating which suggests that two different types of arylsulphatases occur in Nature. Dodgson (1956) and Dodgson & Spencer (1957*a*) have tentatively suggested that those arylsulphatases which show considerable affinity and activity towards simple arylsulphates such as potassium *p*-nitrophenyl sulphate (NPS) and potassium *p*-acetylphenyl sulphate (APS) should be classified as type I arylsulphatases. This type of enzyme is strongly inhibited by cyanide but is not appreciably affected by sulphate or phosphate. On the other hand, those enzymes which show relatively little affinity and activity towards NPS and APS but considerable affinity and activity towards the more complex arylsulphate, dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate, NCS), are classified as type II arylsulphatases. These enzymes are strongly inhibited by sulphate and phosphate but are unaffected by cyanide. Considerable information on the mode of action of one example of a type I enzyme (the bacterial arylsulphatase from *Alcaligenes metalcaligenes*) has already been obtained (Dodgson, Spencer & Williams, 1955, 1956*a*).

Examples of both types of arylsulphatases occur in human liver and other tissues (Dodgson, Spencer & Wynn, 1956*b*). Arylsulphatase C, a type I enzyme, is associated with the microsomes of the liver cell and has not yet been obtained in soluble form. Arylsulphatases A and B are type II enzymes and can readily be obtained in soluble form. The present communication describes the purification and properties of human-liver arylsulphatase B and gives some indication of the nature of the ionizing groups which are involved in the enzymic reaction. A preliminary account of this work has already appeared (Wynn & Dodgson, 1957).

* Part 18: Dodgson & Lloyd (1957).

MATERIALS AND METHODS

Substrates. APS, NPS and potassium phenyl sulphate were prepared by the method of Burkhardt & Lapworth (1926), NCS by the method of Roy (1953) as modified by Dodgson & Spencer (1956*a*) and the monopotassium salts of 4-hydroxy-2-nitrophenyl and 4-hydroxy-3-nitrophenyl sulphates by the method of Smith (1951). The monopotassium salts of *o*-hydroxyphenyl sulphate (catechol monosulphate) and 4-chloro-2-hydroxyphenyl sulphate (4-chlorocatechol monosulphate) were prepared from catechol and 4-chlorocatechol respectively by the method of Burkhardt & Lapworth (1926), but sufficient chlorosulphonic acid was used to sulphate one hydroxyl group only (cf. Dodgson, Rose & Spencer, 1955). Tyrosine *O*-sulphate was prepared by the method of Tallan, Bella, Stein & Moore (1955).

Buffers. In most of the enzyme experiments sodium acetate–acetic acid mixtures (hereafter referred to as acetate buffers) were used to control pH. However, in certain experiments where the properties of the human enzyme were being compared with those of the corresponding enzyme of ox liver, substrate (NCS) solutions were buffered with the acetate–HCl mixture (hereafter referred to as acetate–HCl buffer) described by Roy (1953, 1954). In preparing these solutions Roy used HCl to overcome the strong buffering action of the substrate, but when substrate solutions of varying concentration were required it automatically followed that different amounts of HCl had to be introduced in order to achieve the same pH.

Determination of enzyme activity. Enzyme activity towards APS, NPS and NCS was measured by spectrophotometric estimation of the respective liberated phenols (Dodgson, Spencer & Thomas, 1955). A similar method was