(calcd. C, 57.65; H, 5.38; CH_3O, 11.03. Found: C, 57.62; H, 5.65; CH_3O, 11.14). The presence of a free phenolic hydroxyl group is indicated by a positive ferric chloride reaction, the color being brownish-red in aqueous solution and green in alcoholic solution. The ultraviolet spectrum exhibits a maximum at 286 mμ (log ε 3.66).

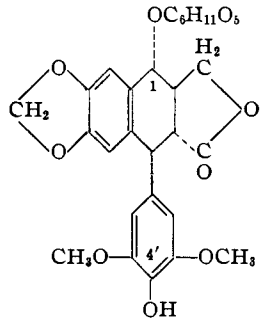
Acetylation of the amorphous glucoside with acetic anhydride in pyridine yields a crystalline pentaacetyl derivative, which melts at 167–169° and exhibits a specific rotation $[\alpha]^{20}D$ −77° in chloroform. The analysis corresponds to an empirical formula $C_{37}H_{40}O_{18}$ (calcd. C, 57.51; H, 5.22; O, 37.27; CH_3O, 8.03; CH_3CO, 27.85. Found: C, 57.22; H, 5.24; O, 37.02; CH_3O, 8.09; CH_3CO, 26.94). The peracetyl derivative gives no coloration with ferric chloride. The ultraviolet spectrum exhibits a maximum at 292 mμ (log ε 3.64) and closely resembles that of podophyllotoxin.

Like podophyllotoxin glucoside, the new glucoside is very sensitive to alkali. In the presence of traces of alkali it undergoes rearrangement to a compound which crystallizes in needles melting at 274–278° and has a specific rotation $[\alpha]^{20}D$ −16° in pyridine. This product has the same empirical formula $C_{27}H_{30}O_{13}$ as the starting material (calcd. C, 57.65; H, 5.38; CH_3O, 11.03. Found: C, 57.49; H, 5.57; CH_3O, 10.73). This rearrangement is analogous to that which occurs in the case of podophyllotoxin glucoside, the product in this case being 4′-demethylpicropodophyllin β-D-glucoside. Here, too, the rearrangement is accompanied by a very pronounced decrease in the solubility in water and in organic solvents.

Hydrolysis of the new glucoside to the aglucone and sugar fraction is readily effected by treatment with β-glucosidase, and no rearrangement occurs. The aglucone agreed in all its properties with the 4′-demethylpodophyllotoxin isolated by Hartwell, *et al.*,[2] from the resin of *P. emodi*. The compound crystallizes from alcohol in needles melting at 250–252° and has a specific rotation $[\alpha]^{20}D$ −131° in chloroform. Our analysis confirmed the formula $C_{21}H_{20}O_8$ (calcd. C, 63.00; H, 5.04; O, 31.97; CH_3O, 15.50. Found: C, 63.03; H, 5.28; O, 31.80; CH_3O, 15.62). The aglucone was characterized by preparation of the diacetate $C_{25}H_{24}O_{10}$ (m.p. 230–231°; $[\alpha]^{20}D$ −130° in chloroform) and the picro derivative (m.p. 218–220°; $[\alpha]^{20}D$ +9° in acetone). The properties of these new compounds are in agreement with those given in the literature.[1,2]

The sugar obtained on enzymatic cleavage could be identified as D-glucose in the form of the α-methyl-D-glucoside <1,5>.

A further proof of the constitution of the new glucoside is provided by the fact that methylation of the free phenolic hydroxyl group with diazomethane converts it into podophyllotoxin glucoside, only a very small portion of the substance undergoing rearrangement to the picro isomer of podophyllotoxin glucoside. The main reaction product, which is obtained only in an amorphous condition, is identical in all its properties with the podophyllotoxin glucoside described in our first communication.



I, 4′-Demethyl-podophyllotoxin β-D-glucoside

The results of our analysis and of the degradation reactions indicate that the new glucoside has the formula $C_{27}H_{30}O_{13}$ and may be designated as 1-O-(β-D-glucopyranosyl)-4′-demethyl-podophyllotoxin (I). Details of the preparation and properties of the substance will be published shortly in *Helvetica Chimica Acta*.

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THE FORMATION OF L-XYLULOSE IN MAMMALS AND ITS UTILIZATION BY LIVER PREPARATIONS[1]

Sir:

The increasing importance of the keto sugars ribulose and sedoheptulose in metabolic studies[2,3] has stimulated interest in the biochemistry of L-xylulose, a sugar whose only known natural occurrence has been in the urine of humans with the genetic biochemical defect usually referred to as essential pentosuria. D-Glucuronolactone has been reported to induce the excretion of increased amounts of the pentose in pentosuric subjects without causing pentose excretion in normal individuals.[4] We wish to report that normal humans and guinea pigs produce L-xylulose from glucuronolactone, that one "non-pentosuric" subject excretes very small amounts of the pentose on a normal diet, and that the pentose is metabolized rapidly by guinea pig liver slices and homogenates.

To isolate L-xylulose from urine, deionized samples were fractionated on Dowex 1 (borate) columns,[5] the Dische cysteine-carbazole[6] test being used to assay eluates. After removal of sodium borate, the eluates were analyzed by paper chromatography. Xylulose was identified on the papergrams by means of anthrone[7] and naphthoresorcinol[8] reagents. Ribulose[9] was ruled out on the

(1) The work described in this paper was supported by grants from the National Science Foundation, The Williams-Waterman Fund of the Research Corporation, and Eli Lilly and Company.

(2) J. A. Bassham, A. A. Benson, L. D. Kay, A. Z. Harris, A. T. Wilson, and M. Calvin, THIS JOURNAL, **76**, 1760 (1954).

(3) B. L. Horecker, M. Gibbs, H. Klenow and P. Z. Smyrniotis, *J. Biol. Chem.*, **207**, 393 (1954).

(4) M. Enklewitz and M. Lasker, *ibid.*, **110**, 443 (1935).

(5) S. Mitsuhashi and J. O. Lampen, *ibid.*, **204**, 1011 (1953).

(6) Z. Dische and E. Borenfreund, *ibid.*, **192**, 583 (1951).

(7) R. Johanson, *Nature*, **172**, 956 (1953).

(8) J. L. Bryson and T. J. Mitchell, *Nature*, **167**, 864 (1951).

(9) We are indebted to Dr. B. L. Horecker for a sample of D-ribulose o-nitrophenylhydrazone.

(2) M. V. Nadkarni, J. L. Hartwell, P. B. Maury and J. Leiter, THIS JOURNAL, **75**, 1308 (1953).

basis of its different color response in the latter test and its different rate of migration in 88% phenol and in butanol–pyridine–water (10:3:3).[10]

The urine of normal guinea pigs and of three normal humans was free of xylulose. The urine of a fourth subject yielded the pentose, as indicated by spectrophotometric examination of the colors obtained in the orcinol[5] and cysteine-carbazole tests, as well as by paper chromatographic comparisons in several different solvents. The pentose was excreted by all four subjects after the ingestion of 17 g. of D-glucuronolactone per day and by guinea pigs fed 0.1 g. of D-glucuronolactone per day. The amounts excreted were approximately 25 to 50 mg. and 1 to 5 mg., respectively. To demonstrate conclusively that the pentose being studied was actually L-xylulose, L-xylosazone (m.p. 161–162.5°) was prepared from a chromatographically purified fraction of the urine of the subject who normally excreted the sugar, glucuronolactone having been fed to increase the amount of pentose excreted. Recrystallization of this product, after it was mixed with an equal amount of pure D-xylosazone, yielded the characteristic crystals of DL-xylosazone (m.p. 198–198.5°).[4]

In contrast to normal humans, pentosurics usually excrete 1.5 to 2.0 g. of L-xylulose daily and twice this amount after ingesting only 5 g. of D-glucuronolactone.[11] It is possible that the pentose is a normal metabolite which is utilized poorly by pentosurics. We are endeavoring to obtain information bearing on this hypothesis. No L-xylulose could be found in the urine of a mouse which had been given intraperitoneally 50 mg. of the pentose (equivalent to 100 g. in a human being).[12] The guinea pig liver experiments were based on similar studies on D-fructose.[13] The liver slices utilized L-xylulose more rapidly than D-ribose or any other pentose tested, and the rate was equal to that of fructose. Homogenates also caused rapid disappearance of the pentose. Unlike fructose, however, the utilization was inhibited by fluoride, and it did not require the addition of adenosine triphosphate if L-glutamate was present.

(10) L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 4047 (1952).

(11) These values are given in reference 4, and we have confirmed them by chromatographic analyses.

(12) The mouse was chosen for this experiment because of the limited quantity of L-xylulose available. It was isolated from the urine of a pentosuric individual (G. W.), to whom we are indebted for supplying us with urine and for participating in experiments involving glucuronolactone.

(13) C. S. Vestling, A. K. Mylroie, U. Irish and N. K. Grant, *J. Biol. Chem.*, **185**, 789 (1950).

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CARBOXYPEPTIDASE, A ZINC METALLOPROTEIN

Sir:

We have recently examined several crystalline preparations of pancreatic (bovine) carboxypeptidase utilizing quantitative emission spectrography. Analyses were performed in duplicate with a porous cup spark excitation system. A Jarrel-Ash varisource in conjunction with a 21-foot Wadsworth spectrograph having a plate factor of 5.18 Å per mm. in the first order was employed.[1] Qualitative and quantitative spectrographic analyses of several crystalline preparations uniformly indicated the presence of large quantities of zinc, while all other metals were either completely absent or present in minute quantities. Table I gives typical quantitative data on a six times recrystallized preparation, prepared by one of us (H. N.),[2] having maximum enzymatic activity toward the substrate carbobenzoxyglycyl-L-phenylalanine[3] and free of proteolytic activity toward substrates of other enzymes (chymotrypsin and trypsin); 1820 micrograms of zinc per g. dry weight of protein were found spectrographically. Zinc measurements were independently confirmed in this and all other analyses (see Table I) by means of a microchemical technique previously described[4] utilizing diphenylthiocarbazone as a colorimetric agent. The molecular weight of carboxypeptidase has been determined[5,6] to be 34,300 and the mole ratio of zinc/carboxypeptidase, for this preparation, is, therefore, 0.96, indicating 1 mole of zinc per mole of carboxypeptidase. Other preparations, from our own and commercial sources, have given similar zinc and other metal distributions and molar ratios.

It will be noted that the amounts of barium, used in the extraction of the enzyme, are minimal in the crystalline preparation. Lithium, employed in recrystallization, could not be detected. All other alkali metals and alkaline earths, including beryllium, magnesium and calcium, were either completely absent or did not exceed a maximum concentration of 5.8 micrograms per gram of protein. In one preparation, no magnesium could be detected at all, either by this or a flame spectrophotometric method.

TABLE I

EMISSION SPECTROGRAPHIC ANALYSIS OF SIX TIMES RECRYSTALLIZED CARBOXYPEPTIDASE

Element	Line / internal standard	μg per g. of carboxypeptidase
Zinc	Zn 3345/V 4111	1820.00
Zinc	By diphenylthiocarbazone	1820.00
Copper	Cu 3247/Bi 3036	33.0
Iron	Fe 3020/V 3185	39.5
Aluminum	Al 3961/V 3185	5.80
Magnesium	Mg 2779/Bi 2897	5.70
Calcium	Ca 4318/Bi 2897	2.65
Barium	Ba 4554/V 3185	18.4

Not found: Beryllium, boron, cadmium, chromium, cobalt, lead, lithium, manganese, molybdenum, nickel, phosphorus, potassium, silver, strontium, tin.

Quantitative analyses for zinc and specific activity during an entire fractionation process have shown a proportionate enrichment of both in the very fractions in which carboxypeptidase is known and was shown to be concentrated. Significantly,

(1) B. L. Vallee, in preparation for publication.

(2) *Cf.* H. Neurath, E. Elkins and S. Kaufman, *J. Biol. Chem.*, **170**, 221 (1947).

(3) E. Elkins-Kaufman and H. Neurath, *ibid.*, **175**, 893 (1948).

(4) B. L. Vallee and J. G. Gibson, 2nd, *ibid.*, **176**, 435 (1948).

(5) F. W. Putnam and H. Neurath, *ibid.*, **166**, 603 (1946); E. L. Smith, D. M. Brown and H. T. Hanson, *ibid.*, **180**, 33 (1949).

(6) E. L. Smith and A. Stockell, *ibid.*, **207**, 501 (1954).