luloses. In the case of coniferous woods the highest values were obtained by application of a mild bisulfite treatment[1,2] which must, however, have involved some degradation. Nitration with a mixture of nitric acid, phosphoric acid and phosphorus pentoxide is known to have little or no degrading effect on carbohydrates[3] and this method, when applied to two deciduous wood species,[1,4] gave D.P. values of 2900 to 3000. The same procedure, when used on coniferous woods, however, resulted in either incomplete nitration or excessive degradation.[1,5]

In connection with a study of the carbohydrates of balsam fir (*Abies balsamea*) it has now been found that the cellulose component of this coniferous wood can be isolated by a direct nitration procedure in almost quantitative yield and with apparently no degradation provided the time of nitration is sufficiently extended. Wood meal was treated for various lengths of time with standard nitric acid, phosphoric acid and phosphorus pentoxide mixture[3] at 17° and the cellulose nitrate was isolated by methanol washing and precipitation from acetone, as used by Bryde and co-workers.[6] The degrees

TABLE I

NITRATION OF BALSAM FIR WOOD

Time, hr.	Yield,[a] %	Cellulose in wood,[b] %	D.P.
12	132.9		2960
18	122.2		2960
36	116.0		3350
48	110.9	48.8	3200
58	110.9	48.2	3000
76	110.2	48.8	2900
82	110.5	48.8	3100

[a] Nitrated wood, based on extractive-free, oven-dry wood. [b] Yield of cellulose nitrate in %/1.80.

of polymerization were calculated from the relationship D.P. = $80[\eta]^3$ where $[\eta]$ was the intrinsic viscosity of the nitrate in ethyl acetate. The values given in Table I are somewhat scattered but indicate, contrary to earlier findings, that no degradation occurred during the nitration, the D.P. actually being the same after 6 and 100 hr. A maximum amount of wood was apparently nitrated after 48 hr. Based on the yield and an average nitrogen content of 13.72% of the cellulose nitrate, the cellulose content of the original wood was calculated to 48.8%, a value that corresponded well with the α-cellulose content of the wood, or 49.4%, thus indicating that the true cellulose was almost completely accounted for.

For a more accurate determination of the chain length use was made of the relationship developed by Newman, Loeb and Conrad[7] between the intrinsic viscosity of a cellulose nitrate at a rate of shear of 500 sec.$^{-1}$ and its D.P. as obtained from sedimentation-diffusion measurements. The reduced viscosity in ethyl acetate was estimated in a viscometer permitting variation of the rate of shear.[8] After correction for kinetic energy losses and graphic extrapolation to zero concentration, intrinsic viscosity values were obtained ranging from 38.8 dl./g. at a shear rate of 2000 sec.$^{-1}$ to 44.5 at 200 sec.$^{-1}$. The value at 500 sec.$^{-1}$, 42.5, after correction for its nitrogen content,[9] was 40.9, corresponding to a D.P. of 3270.[7] Although this seems to be the highest D.P. value so far noted for a wood cellulose, it falls far below those found by a similar procedure for native flax, cotton and ramie fibers which were all within the range of 5000 to 6000.[10]

A complete account of this work will be given later.

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THE ISOLATION OF 4'-DEMETHYL-PODOPHYLLOTOXIN-GLUCOSIDE FROM RHIZOMES OF *PODOPHYLLUM EMODI* WALL.

Sir:

In a previous communication,[1] we reported the isolation of podophyllotoxin β-D-glucoside from the Indian plant *Podophyllum emodi* Wall. This glucoside is present in the drug to the extent of 0.5 to 1%; its aglucone occurs in larger quantities in the resin fraction of the rhizomes and is responsible for the antimitotic effect of podophyllin resin and its influence on the growth of tumors. The glucoside is an amorphous substance which has a solubility in water of approximately 2% and which is rapidly broken down to podophyllotoxin and D-glucose by β-glucosidase.

From the mother liquors resulting from the purification of podophyllotoxin glucoside, it was possible to isolate a further glucoside. This previously unknown compound gives a positive reaction with ferric chloride and is more soluble in water than podophyllotoxin glucoside. It is present in the dried rhizomes of *P. emodi* to the extent of approximately 0.2 to 0.5%, and has been identified as 4'-demethylpodophyllotoxin β-D-glucoside (I).

Like the glucoside of podophyllotoxin, the glucoside of demethylpodophyllotoxin shows no tendency to crystallize and is isolated as a light, white powder. It melts at 165–170° and has a specific rotation $[\alpha]^{20}D$ $-75°$ in water, $[\alpha]^{20}D$ $-81°$ in methanol and $[\alpha]^{20}D$ $-123°$ in pyridine. The analysis corresponded to an empirical formula $C_{27}H_{30}O_{13}$

(1) A. Stoll, J. Renz and A. von Wartburg, THIS JOURNAL, **76**, 3103 (1954).

(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.

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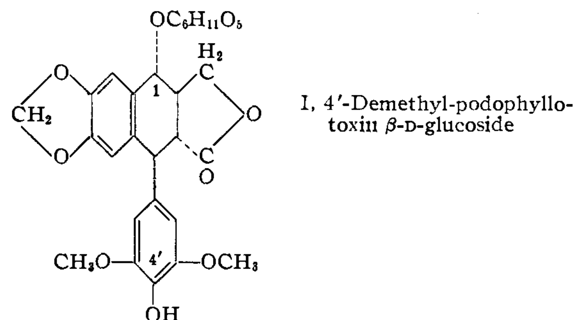

I, 4'-Demethyl-podophyllotoxin β-D-glucoside

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.

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