that a new enzyme, "isocitritase," catalyzes the reaction

Isocitric acid ⟶ glyoxylic acid + succinic acid (1)

The conditions for growing the cells, preparing the extracts, and separating aconitase from isocitritase are shown in Table I. Isocitrate is the substrate

TABLE I

SEPARATION OF ISOCITRITASE FROM ACONITASE

Reaction 10 minutes, 30° under nitrogen in 3-ml. containing: 200 μM. tris (tris-(hydroxymethyl)-aminomethane) buffer *p*H 7.6; 10 μM. $MgCl_2$; 10 μM. glutathione; 20 μM. DL-isocitrate; reaction started with enzyme (0.5 to 5 units), stopped with 0.3 ml. 100% TCA.

	Fraction	Absorption ratio 280/260	Protein g.	Isocitritase[b] units × 10^3	recov. %	Aconitase[c] units × 10^5
1	Extract[a]	0.57	2.3	6.46	100	12.4
2	$AmSO_4$-1 0.25–0.88 satd.	0.58	1.8	3.8	59	
3	2 + protamine	0.93	1.6	2.9	44	
4	$AmSO_4$-2 0.43–0.62 satd.	1.49	0.46	2.7	42	nil

[a] *P. aeruginosa*, ATCC 9027, was grown with aeration in an acetate mineral salts medium; 22 g. of cell paste, suspended in 200 ml. of *M*/50 phosphate buffer *p*H 7.0 containing 50 mg. of glutathione, was oscillated 15 min. in a 10 KC. Raytheon, and centrifuged 1 hour at 16,000 × *g* to obtain an extract. [b] 1 unit = 1 μM. glyoxylate formed per 10 min. in protocol above. [c] 1 unit = 0.001 OD increase/min. at 240 mμ; ≈ 0.05 μM. aconitate accumulated/hour/3 ml. cuvette: Racker, *Biochim. et Biophys. Acta*, **4**, 211 (1950).

of this enzyme, as shown by the data in Table II; *i.e.*, with crude preparations all three tricarboxylic acids yield glyoxylate, whereas after the removal of aconitase only isocitrate serves as substrate. The stoichiometry of the reaction with both crude and fractionated extracts is also indicated in Table II.

TABLE II

STOICHIOMETRY AND SUBSTRATE SPECIFICITY OF ISOCITRITASE

Reaction as in Table I except run 20 minutes.

Fraction and substrate	μM.	Used μM.	Products formed Glyoxylate[b] μM	Succinate[c] μM
Extract, 1.1 mg. P				
DL-isocitrate,	20	4.14[a]	3.64	4.18
cis-aconitrate,	12.5		2.24	
citrate	12.5		0.72	
Isocitritase (Fr. 4, Tbl. I, 0.75 mg P)				
DL-isocitrate,	20	4.54	4.81	4.58
cis-aconitate,	12.5		nil	
citrate	12.5		nil	

[a] D-Isocitric acid determined by method of Ochoa in J. B. Sumner and K. Myrback, "The Enzymes," Academic Press, Inc., New York, N. Y., 1952, Vol. II, p. 1017. [b] Glyoxylate determined by the method of Friedemann and Haugen, *J. Biol. Chem.*, **147**, 415 (1943), using crystalline glyoxylic acid 2,4-dinitrophenylhydrazone as standard. [c] Succinate measured with succinoxidase according to Dietrich, *et al.*, *Arch. Biochem.*, **41**, 118 (1952).

Early fractionations revealed requirements for a divalent metal and for sulfhydryl as activators. Dialysis of Fraction 4, Table I, against *M*/50 Versene *p*H 7.4 for 20 hours at 4°, followed by 14 hours dialysis *vs.* *M*/50 KCl at the same temperature, rendered the isocitritase completely dependent on magnesium ion and a sulfhydryl compound, as indicated by the saturation curves in Fig. 1. Glutathione and cysteine were equally effective activators for isocitritase at all stages of enzyme purity so far studied. Ferrous and cobaltous ions were 40% as active, and manganous about 20% as active, as magnesium.

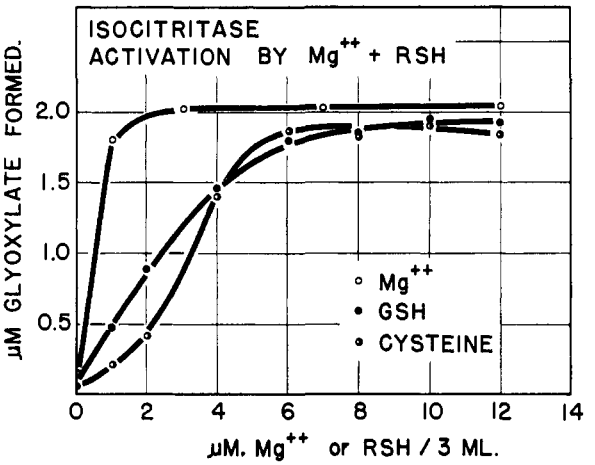

Fig. 1.—Magnesium and sulfhydryl activation of isocitritase: reaction 10 minutes, 30° under nitrogen in 3 ml. containing: 200 μM. tris buffer, *p*H 7.6; 3 μM. $MgCl_2$ or as indicated; 10 μM. RSH or as indicated; enzyme Fr. 4, table I ≈ .38 mg. protein; reaction started with 20 μM. DL-isocitrate; stopped with 0.3 ml. 100% TCA.

Isocitritase does not require Coenzyme A, as indicated by retention of full activity after Dowex treatment,[4] which completely removed the Coenzyme A from the extracts, as indicated by transacetylase assay.[5]

Although the reaction does not proceed to completion, and succinate inhibits the forward reaction, our experiments have not so far demonstrated a reversibility either with crude or fractionated preparations unsupplemented, or upon the addition of ATP or other activators. Further data will be required to clarify this point.

The isocitritase reaction (1) constitutes an aldolase cleavage of isocitric acid analogous to the inducible citritase, or citridesmolase, which cleaves citrate to oxalacetate and acetate, as previously described in *Streptococcus faecalis*[6] and *Escherichia coli*.[7]

(4) H. Chantrenne and F. Lipmann, *J. Biol. Chem.*, **187**, 757 (1950).
(5) E. R. Stadtman, G. D. Novelli and F. Lipmann, *ibid.*, **191**, 365 (1951).
(6) D. C. Gillespie and I. C. Gunsalus, *Bact. Proc.*, **80**, (1953).
(7) M. Grunberg-Manago and I. C. Gunsalus, *ibid.*, **73** (1953), and *J. Bact.*, 1954, in press.

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THE DEGREE OF POLYMERIZATION OF THE CELLULOSE COMPONENT OF BALSAM FIR

Sir:

Many attempts have been made to establish the degree of polymerization (D.P.) of native wood cel-

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.

(1) L. Jörgensen, "Studies on the Partial Hydrolysis of Cellulose," Emil Moestue A/S, Oslo, 1950, pp. 11-22; E. Heuser and L. Jörgensen, *Tappi*, **34**, 57 (1951).

(2) Ö. Bryde and B. Råny. "Svensk Papperstidn.," Hägglund Issue, June 15, 1947, p. 34.

(3) W. J. Alexander and R. L. Mitchell, *Anal. Chem.*, **21**, 1487 (1949).

(4) T. E. Timell and E. C. Jahn, *Svensk Papperstidn.*, **54**, 831 (1951).

(5) R. L. Mitchell, *Ind. Eng. Chem.*, **38**, 843 (1946).

(6) Ö. Bryde, *Svensk Papperstidn.*, **52**, 389 (1949); F. A. Abadie nd Ö. Ellefsen, *Norsk Skogind.*, **6**, 192 (1952); Ö. Ellefsen, *ibid.*, **7**, 05 (1953).

(7) S. Newman, L. Loeb and C. M. Conrad, *J. Polymer Sci.*, **10**, 463 (1953).

(8) J. Schurz and E. H. Immergut, *ibid.*, **9**, 279 (1952).

(9) C. H. Lindsley and M. B. Frank, *Ind. Eng. Chem.*, **45**, 2491 (1952).

(10) T. E. Timell, unpublished data.

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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).