

ISOLATION OF GLUTATHIONE FROM POTATO TUBERS TREATED WITH ETHYLENE CHLOROHYDRIN¹

Sir:

Treatment of freshly-harvested potato tubers with ethylene chlorohydrin, a chemical that breaks their dormancy,² increases the sulfhydryl content of the expressed juice as shown by the nitroprusside test and by the sulfur reduction method.³ In order to find whether this increase in sulfhydryl was due to an increase in glutathione, the procedure of Hopkins⁴ for the isolation of glutathione was applied to the juice of potatoes treated with ethylene chlorohydrin.

Cut pieces of freshly-harvested potato tubers were dipped in a 1.6% solution of ethylene chlorohydrin, the excess solution drained off and the pieces placed in a closed container for twenty-four hours. They were then planted in moist soil for five days. After washing free of soil, the pieces were peeled, ground through a food chopper, the juice was expressed through cheesecloth, and the starch centrifuged out. The juice was boiled to coagulate the proteins, cooled, and filtered through glass wool. Following the procedure of Hopkins the cuprous salt of glutathione was isolated. In one experiment 1.5 liters of the boiled, filtered juice yielded 870 mg. The copper content was 17.48%; calcd., 17.26%.

The cuprous salt prepared by the above method was suspended in water and decomposed with hydrogen sulfide. After filtering out the cupric sulfide, the hydrogen sulfide was removed in a stream of hydrogen and the filtrate evaporated in a vacuum desiccator over sulfuric acid. When reduced to small volume, absolute alcohol was added and the glutathione allowed to crystallize. The yield from 395 mg. of the cuprous salt was 190 mg. of crystalline glutathione. An additional 70 mg. was obtained by bringing the mother liquor to dryness. The crystalline fraction was analyzed for nitrogen (micro Kjeldahl) and sulfur (Parr bomb).

Anal. Subs., 23.04 mg.: 10.8 cc. of *N*/50 H₂SO₄. Subs., 0.1141 g.: 0.0862 g. BaSO₄. Calcd. for C₁₀H₁₇N₃SO₆: N, 13.7; S, 10.42. Found: N, 13.1; S, 10.38.

By the same procedure, 970 mg. of the cuprous salt yielded 650 mg. of glutathione. This was recrystallized and the first fraction, which weighed 275 mg., was analyzed.

Anal. Subs., 30.99 mg.: 14.7 cc. of *N*/50 H₂SO₄. Subs., 0.1024 g.: 0.0771 g. BaSO₄. Found: N, 13.3; S, 10.34.

Using the same procedure, no glutathione could be isolated from the juice of untreated tubers, although qualitative tests indicated that a small

¹ Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 36.

² Denny, *Am. J. Botany*, **13**, 118 (1926).

³ Guthrie and Wilcoxon, *Contrib. Boyce Thompson Inst.*, **4**, 99 (1932).

⁴ Hopkins, *J. Biol. Chem.*, **84**, 269 (1929).

amount was present. When potatoes that had been stored for several months were treated with ethylene chlorohydrin, the cuprous salt could be isolated, but in smaller yield than with freshly-harvested tubers. In one experiment, 1 liter of juice yielded 280 mg. of the cuprous salt; in another 600 cc. yielded 60 mg. The copper content of the latter preparation was 17.15%.

This is believed to be the first isolation of glutathione from a seed plant.

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E. L. HIRST'S COMMENTS ON IRVINE AND MCGLYNN'S PAPER ON
"A POSSIBLE SOURCE OF ERROR IN DETERMINING THE CONSTITUTION
OF DI- AND POLYSACCHARIDES"¹

Sir:

The criticisms made by Dr. E. L. Hirst reveal that in his reading of the paper to which he refers he has overlooked the essential features of the work described. Constitutions are not assigned to di- and polysaccharides but attention is directed, systematically and on the evidence of experimental work described for the first time, to possible sources of error in determining the constitution of these compounds by the methylation method. It is the case that the possibility of 2,3,6-trimethylglucose functioning in both a normal and a γ -form has already been suggested but our omission of any reference to Schlubach and Bomhard [*Ber.*, 59, 845 (1926)] does not involve that we overlooked previous work on the subject. It is at least unlikely that, as the originator of the method of solving the constitution of di- and polysaccharides by methylation, the efforts of other workers in this field should have been overlooked by me. But it may be pointed out that Schlubach and Bomhard merely converted the sugar into tetramethyl- γ -glucose through the intermediate formation of an unpurified glucoside, while in our case this glucoside was isolated after vacuum distillation as a pure compound, was analyzed and, among other reactions, was subjected to the graded hydrolysis which is obviously an essential part of the experimental scheme. The tautomerism of 2,3,6-trimethylglucose is in consequence no longer conjectural but is based on experimental evidence.

With regard to his criticisms of our studies on the oxidation of trimethylglucose, Dr. Hirst assumes that, when this reaction is carried out in the presence of calcium carbonate under the conditions we describe, the whole of the acid produced is transformed into the calcium salt. Such is

¹ Irvine and McGlynn, *THIS JOURNAL*, 54, 358 (1932).