Phytochemistry, 1969, Vol. 8, pp. 1405 to 1406. Pergamon Press. Printed in England.

SHORT COMMUNICATION

THE USE OF THIOGLYCOLLATE FOR EXTRACTING PHENYLBORONIC ACID FROM ROOTS OF BEAN SEEDLINGS

R. A. WILDES, J. W. ANDERSON,* K. S. ROWAN and T. F. NEALES

Botany School, University of Melbourne, Parkville, Victoria, 3052, Australia

(*Received* 14 *December* 1968)

Abstract—Phenylboronic acid is rendered insoluble in diethyl ether during extraction from roots of the bean (*Vicia faba* L.) by binding with tannins. Including thioglycollate in the extracting medium inhibits both enzymic and non-enzymic formation of tannins and results in recovery of phenylboronic acid in the ether phase.

QUINONES formed by enzymic oxidation of endogenous phenolics and the tannins formed by condensation of these quinones[1] inactivate both soluble and particulate enzymes during extraction from plant tissue.[2,3] In this paper we report that phenolic oxidation-products also interfere with extraction of phenylboronic acid from bean roots and describe methods of overcoming this interference by the use of thioglycollate.

RESULTS

The obligate boron requirement of plants is usually satisfied by addition of boric acid to the root medium. However phenylboronic acid will replace boric acid as a boron source.[4] When roots of *Vicia faba* were treated with phenylboronic acid, extracts prepared from the treated roots by the method of Torsell[5] became brown after maceration of the tissue in water and turned black when the pH of the boiled extracts was raised to 10·5, presumably through both enzymic and non-enzymic oxidation of phenolics.[6] When we prepared extracts from roots, previously treated with unlabelled phenylboronic acid, using an extracting medium containing [^{14}C]phenylboronic acid, only 0·8 per cent of the radioactivity added was recovered on subsequent extraction with diethyl ether. Since we suspected that the tannins formed during extraction and subsequent treatment at high pH were binding phenylboronic acid in a form insoluble in ether, we examined the effects of adding thioglycollate or metabisulphite to the extracting medium at various stages of the extraction (Table 1).

Adding thioglycollate to the extract before raising the pH to 10·5 was the most effective single treatment; adding thioglycollate to the extracting medium followed by a second addition of thioglycollate to the extract before adjusting the pH to 10·5 gave the highest recovery of label and was more effective than adding metabisulphite to the extracting medium.

* Present address: Botany Department, University College London, Gower Street, London, W.C.1, England.

[1] T. SWAIN, in *Plant Biochemistry* (edited by J. BONNER and J. E. VARNER), p. 552, Academic Press, New York (1965).
[2] W. D. LOOMIS and J. BATTAILE, *Phytochem.* **5**, 423 (1966).
[3] J. W. ANDERSON, *Phytochem.* **7**, 1973 (1968).
[4] C. ODHNOFF, *Physiol. Plantarum* **14**, 187 (1961).
[5] K. TORSSELL, *Physiol. Plantarum* **16**, 92 (1963).
[6] S. A. GORDON and L. G. PALEG, *Plant Physiol.* **36**, 838 (1961).

TABLE 1. EFFECT OF POTASSIUM METABISULPHITE AND SODIUM THIOGLYCOLLATE ON THE RECOVERY OF [U-^{14}C]PHENYLBORONIC ACID ADDED IMMEDIATELY BEFORE MAKING EXTRACTIONS FROM SAMPLES OF BEAN ROOTS

Additions to extracting medium*	Further additions to extract prior to raising pH	Recovery of label in ether phase (%)
Nil	Nil	5·4
Nil	Sodium thioglycollate (100 mM)	75·4
Potassium metabisulphite (5 mM)	Nil	9·8
Potassium metabisulphite (5 mM)	Sodium thioglycollate (100 mM)	89·8
Sodium thioglycollate (50 mM)	Sodium thioglycollate (50 mM)	95·7

* 4·9 μMoles of [U-^{14}C]phenylboronic acid was added to 9·1 g of roots. Extractions were made at pH 5.

We concluded that thioglycollate inhibited non-enzymic oxidation of phenolics and subsequent condensation and polymerization to tannins and insoluble dark pigments more effectively than metabisulphite at the concentrations used in these experiments. Metabisulphite added to the extracting medium protects tobacco-leaf peptidase,[7] potato-tuber mitochondria[8] and phosphofructokinase (Chalmers and Rowan, unpublished) by inhibiting phenolase.[7] Although much higher concentrations of metabisulphite might prevent tannins binding phenylboronic acid during extraction, 4 mM metabisulphite does not prevent oxidation of aqueous extracts prepared from tobacco leaf when incubated at pH 9, though it prevents oxidation efficiently at pH 5 (Anderson and Wildes, unpublished). Thus loss of reducing power at high pH apparently contributes to failure of metabisulphite to inhibit non-enzymic browning in extracts from bean roots. Extracts prepared from roots previously treated with [^{14}C]phenylboronic acid using thioglycollate in the extracting medium contained considerably more ^{14}C than control extracts, but the ^{14}C had an R_f value (0·9) much higher than authentic [^{14}C-]phenylboronic acid (0·3). However, when the radioactive spot was eluted and treated with 0·5 N HCl at room temperature, part of the radioactivity then ran with R_f value equal to phenylboronic acid. Thus the [^{14}C-]phenylboronic acid fed to the tissue appeared to be bound by an acid-labile bond to another compound.

EXPERIMENTAL

Seeds of bean (*Vicia faba* L., cultivar "Minor") were soaked in H_2O for 12 hr and germinated on "Perlite" for 48 hr. They were then grown at 25° with roots immersed in a solution of boric acid (0·5 mM) and Ca $(NO_3)_2$ (1·25 mM) for 24 hr before treating with quarter strength Hoagland's nutrient medium No. 1 for a further 41 hr. Radicles were excised from bean seedlings, washed and blended in 3 vol. of distilled H_2O containing 4·9 μmoles [U-^{14}C]phenylboronic acid (specific activity: 1·2 μc/mmole), using a M.S.E. "Homogenizer". The extracts were boiled for 10 min and the supernatant solutions recovered by centrifugation. The precipitates were re-extracted with H_2O and boiled twice more and the supernatant solutions combined (approx. 100 ml). The extracts were adjusted to pH 10·5 with 5 N-NaOH and concentrated in a rotary film evaporator until the volume was reduced to 10–15 ml. The pH was then lowered to 4·5 with 11 N-HCl and extracted four times with Et_2O. The Et_2O phase was evaporated to dryness in scintillation vials in a hot-water bath. Radioactivity was counted (counting efficiency 69 per cent) using 2,5-diphenyloxazole and 1,4-di-2 [4 methyl-5-phenyloxazolyl] benzene dissolved in toluene.

Acknowledgements—The [U-^{14}C]phenylboronic acid was a gift from Borax Consolidated Ltd. Research Laboratories, Chessington, England. This work was supported by grants from the Rural Credits Development Fund and the Central Tobacco Advisory Committee.

[7] J. W. ANDERSON and K. S. ROWAN, *Phytochem.* **6**, 1047 (1967).
[8] D. M. STOKES, J. W. ANDERSON and K. S. ROWAN, *Phytochem.* **7**, 1509 (1968).