Phytochemistry, 1969, Vol. 8, pp. 2259 to 2260. Pergamon Press. Printed in England.

SHORT COMMUNICATION

AN IMPROVED PROCEDURE FOR THE ISOLATION OF DHURRIN

P. F. REAY

Department of Biochemistry and Biophysics, University of California,
Davis, California 95616

(*Received* 20 *May* 1969)

Abstract—Dhurrin was first isolated in pure crystalline form by Mao *et al.*[1] who reported on its physical properties. A continuing interest in dhurrin biosynthesis along with a need for several grams of dhurrin prompted a development of its isolation on a larger scale. Because phenolic compounds have been isolated using polyvinylpyrrolidone[2] (PVP) for chromatography, this adsorbent was investigated for purifying dhurrin from salts and glucose present in extracts of sorghum.

DHURRIN, was extracted from the shoots of etiolated seedlings of *Sorghum vulgare* since these contain high levels of the glucoside.[3] The extract was freed from lipids by separating it into two phases[4] and some flavonoids were precipitated with neutral lead acetate.[1] It was found that dhurrin could be separated from much of the sugar by extraction into isopropanol and the remainder of the sugar, salts and some substances absorbing light in the u.v. were separated from dhurrin on PVP. In a typical isolation the overall yield of crystalline dhurrin was 61 % of the 1·5 g present in the shoot extract.

EXPERIMENTAL

Shoots (215 g) from 3-day-old etiolated seedlings of *Sorghum vulgare* were placed in 3 l. MeOH : $CHCl_3$: 2 N formic acid[4] (12 : 5 : 3) at −80°. The mixture was stored 24 hr at −20° and then homogenized at room temperature. The homogenate was filtered through "miracloth", the residue was washed with MeOH : $CHCl_3$: H_2O (12 : 5 : 3) and lightly pressed.

Aliquots (600 ml) of the extract were separated into two phases.[4] The bottom phase was extracted with H_2O (1/10 vol), the water added to the top phase and the bottom phase discarded. The solution was filtered through Whatman No. 1, evaporated to a gum on the rotary evaporator, taken up in 75 ml water and filtered through PVP on a sintered-glass filter.

Lead acetate was added to the filtrate until no further precipitate appeared. The solids were filtered off and the lead ions removed with H_2S. The solution was evaporated to a gum and made up to 17 ml with water.

This solution was rapidly stirred while 75 ml isopropanol was slowly added. Two phases separated out and were stirred for an hour. The gummy aqueous phase was allowed to settle and the isopropanol phase containing dhurrin poured off. The gum was rinsed with 20 ml isopropanol and then allowed to stand overnight with a further 20 ml. The combined isopropanol solutions were dried ($MgSO_4$) and this was filtered

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[1] C.-H. MAO, J. P. BLOCHER, L. ANDERSON and D. C. SMITH, *Phytochem.* **4**, 297 (1965).

[2] J. W. MIZELLE, W. H. DUNLAP, R. E. HAGEN, S. H. WENDES, B. J. LIME, R. F. ALBACH and F. P. GRIFFITHS, *Anal. Biochem.* **12**, 316 (1965).

[3] T. AKAZAWA, P. MILJANICH and E. E. CONN, *Plant Physiol.* **35**, 535 (1960).

[4] R. L. BIELESKI and R. E. YOUNG, *Anal. Biochem.* **6**, 54 (1963).

off along with the other insoluble substances. The isopropanol solution was reduced to about 20 ml on the rotary evaporator, the solution decanted from a gum which had formed and the latter extracted for 12 hr with 5 ml isopropanol, which was added to the solution.

About half the isopropanol solution was added to a column (2·1 × 26 cm) of PVP and the column washed with 150 ml isopropanol. Dhurrin was eluted with 300 ml MeOH:isopropanol (30:70) (MI) followed by 1 l. 40:60 MI; both eluting solvents contained 1 drop acetic acid/100 ml. Dhurrin appeared in the effluent after 190 ml MI had entered the column. The fractions containing the bulk of the dhurrin were evaporated to a dry foam on the rotary evaporator.

The foam was dissolved in a minimum of warm BuOH, allowed to crystallize at room temperature overnight and then cooled to 4°. The crystals were rinsed with BuOH and dried *in vacuo*. Found: m.p. 166–168°, mixed, 164–166°; λ_{max}^{HCl}, 230 nm, λ_{max}^{NaOH}, 256 nm, λ_{max} (0·1 N NaOH after 30 min) 330 nm. (Reported: m.p. 164–166°; λ_{max}^{HCl}, 230 nm, λ_{max}^{NaOH}, 255 nm, λ_{max} (0·1 N NaOH after 30 min), 330 nm.)

PVP, washed and dried,[6] was washed with ammonia, methanol, formic acid, methanol and isopropanol. The fines were decanted, the PVP was packed at 5 lb/in^2, and washed with isopropanol. After each use the column was washed with methanol followed by isopropanol.

Dhurrin was estimated from the cyanide released after hydrolysis in centre well flasks for 48 hr with emulsin. The HCN diffused into 1 N NaOH during this time and was determined by the method of Aldridge.[5] Dhurrin in the column effluent was determined spectrophotometrically at 230 nm.

Acknowledgements—I would like to thank Greg Schmidt for his assistance in carrying out this work which was supported by USPHS Grant GM-5301 awarded to E. E. Conn. I also acknowledge the receipt of a fellowship from the University Grants Committee of New Zealand.

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[5] W. N. ALDRIDGE, *Analyst* **69**, 262 (1944).

[6] W. D. LOOMIS and J. BATTAILE, *Phytochem.* **5**, 423 (1966).