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Inulin in Medicinal Plants. I. Determination of Inulin in Medicinal Plants by X-Ray Diffractometry

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An X-ray diffractometric method was developed for the determination of inulin in plants. Dextrin and α-alumina were used as the diluent and internal standard material, respectively. The plant sample was ground to a powder with a propeller-type mill, because the use of a ball mill markedly reduced the intensity of the diffraction line of inulin. The powdered sample was mixed with α-alumina in an agate mortar for diffractometric analysis.

This convenient method (detection limit, 4.0%; standard deviation, 3.9%) was applied to the determination of inulin in some medicinal plants of the Compositae and Campanulaceae families, and high inulin contents were obtained in the cases of plants such as the dahlia, which were thought to contain a large amount of inulin on the basis of microscopic examination.

Keywords——inulin; fructan; X-ray diffractometry; quantitative analysis; internal-standard technique; medicinal plant; Compositae family; Campanulaceae family

Inulin is a fructan (or more correctly a glucofructan) which occurs as a carbohydrate reserve in tubers or roots in some plants of the Compositae and Campanulaceae families. This is of interest in connection with plant physiology because almost all other plants store carbohydrates in their tubers in the form of starch. Examination of inulin contents in various parts of plants according to the vegetational period would provide useful physiological information, and a convenient analysis procedure for inulin in plants is required.

Several workers[2–6] have investigated the determination of inulin in biological fluids to develop a renal clearance test. Jacobsson[5] and Davidson *et al.*[6] reported that inulin in diabetics could be successfully determined by colorimetry combined with gel filtration using Sephadex G-25. However, a procedure for the determination of inulin in plants has not been established due to the more complex matrix, including the presence of fructose, saccharose, and oligo- and polysaccharides.

X-Ray diffractometry is frequently useful for analytical determinations such as that of quartz in the presence of mineral silicates, and the relative amounts of polymorphs in mixtures; these would be difficult or impossible by chemical methods.

In this study, therefore, an attempt was made to apply X-ray diffractometry to the determination of inulin in plants, and some medicinal plants of the Compositae and Campanulaceae families were analyzed.

Experimental

Apparatus——A Rigakudenki Rotaflex (model RU-200) X-ray diffractometer, equipped with a graphite monochromator and a scintillation counter, was used. The operating conditions were as follows; X-ray source, Cu-kα; voltage, 40 kV; current, 150 mA; count range, 4000 cps; angle range, $2\theta=4°$ (interplanar

1) Location: *2-10-65 Kawai Matsubara-City, Osaka 580, Japan.*
2) J.H. Roe, *J. Biol. Chem.*, **107**, 15 (1934).
3) A.S. Alving, J. Rubin, and B.F. Miller, *J. Biol. Chem.*, **127**, 609 (1939).
4) R.M. Reinecke, *J. Biol. Chem.*, **142**, 487 (1942).
5) L. Jacobsson, *Clin. Chim. Acta*, **7**, 180 (1962).
6) W.D. Davidson, M.A. Sackner, and M.H. Davidson, *J. Lab. Clin. Med.*, **62**, 501 (1963).

spacing, $d=22.09$ Å) $-60°$ ($d=1.54$ Å). Measurement of the intensity of X-rays diffracted was based on the peak height.

A Spex mixer mill (ball-type) or a fron Philips coffee mill (propeller-type) was employed for grinding.

Materials——Inulin was obtained from Merck (Germany). All other reagents were of special grade or the highest quality available.

Most of the plant samples were collected on Mt. Kongō from November 15 to December 15, 1977, and the underground parts were used for the measurements. Crude drug samples were purchased from Osaka market.

Results and Discussion

Analytical Line

Figure 1 shows X-ray diffraction patterns of inulin and some typical samples. The 12.0° and 21.0° lines of inulin are rather sharp, and are thus possible analytical lines for inulin. The 21.0° line is unsuitable, however, because all plant samples tested give an X-ray diffraction pattern having a broad peak near 20°. The 12.0° line was therefore used for the analysis.

The sharpness of the 12.0° line of any sample tested is similar to that of inulin (Merck), so the variation of inulin crystallinity in different plant samples seems to be negligible.

Internal Standard Material

Either a direct or an internal-standard technique[7] is generally used for quantitative analysis in terms of the intensity of X-rays diffracted. The latter is suitable for the determination of inulin in plants by X-ray diffractometry, because the mass absorption coefficient of the sample matrix is unknown.

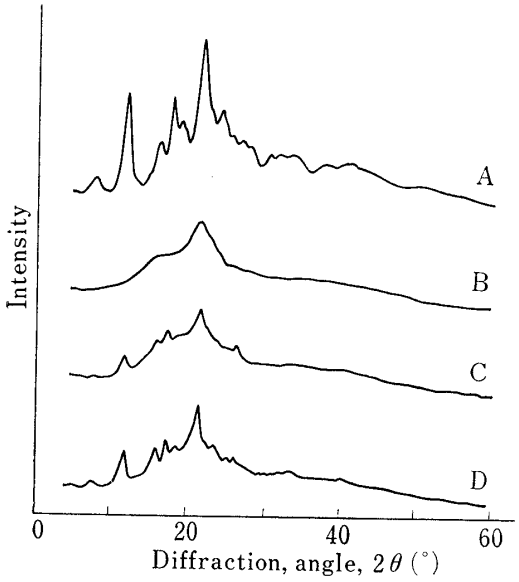

Fig. 1. X-Ray Diffraction Patterns of Inulin and Some Medicinal Plants

A: inulin, B: *Ligularia fischeri* Turcz, C: *Inula helenium* L, D: *Platycodon grandiflorum* A. DC.

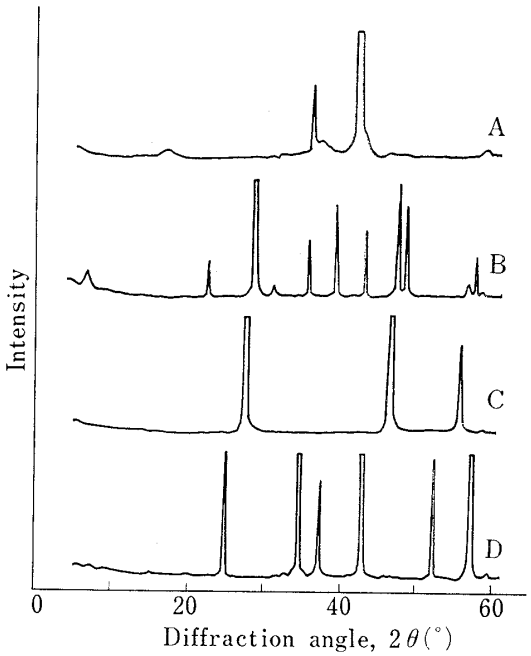

Fig. 2. X-Ray Diffraction Patterns of Materials tested as Internal Standards

A: MgO, B: $CaCO_3$, C: CaF_2, D: α-Al_2O_3.

Figure 2 shows X-ray diffraction patterns of MgO, $CaCO_3$, CaF_2 and α-Al_2O_3, which are frequently used as internal standard materials in X-ray analysis because of their high crystal-

7) *a*) L.E. Alexander and H.P. Klug, "X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials," John Wiley and Sons, Inc., New York, 1974, pp. 532—534; *b*) L. Alexander and H.P. Klug, *Anal. Chem.*, **20**, 886 (1948).

linity and low degree of preferred orientation. Since inulin and all plants tested have no peak and low backgrounds in the diffraction angle range of 50—60°, a substance with a strong peak in this range would be preferred as an internal standard, though a line as close as possible to the analytical line is used in general. In practice, the use of the 25.4° line of α-Al_2O_3 gave less reproducible values than that of its 57.4° line. Therefore, the 57.4° line of α-Al_2O_3 was used as an internal standard analytical line.

A suitable amount of α-Al_2O_3 was found to be about 15% (w/w) of the sample, based on the relative intensity of inulin and α-Al_2O_3 peaks.

Diluent for Calibration Curve

Figures 3 and 4 show X-ray diffraction patterns of materials tested as diluents to obtain a calibration curve and those of mixtures of inulin and α-Al_2O_3 with each diluent, respectively. In the case of $CaCO_3$, though the background near 12° is low in the X-ray diffraction pattern of the pure material, the intensity of the diffraction line of inulin in the pattern of the mixture was reduced. This is due to the high mass absorption coefficient of $CaCO_3$.

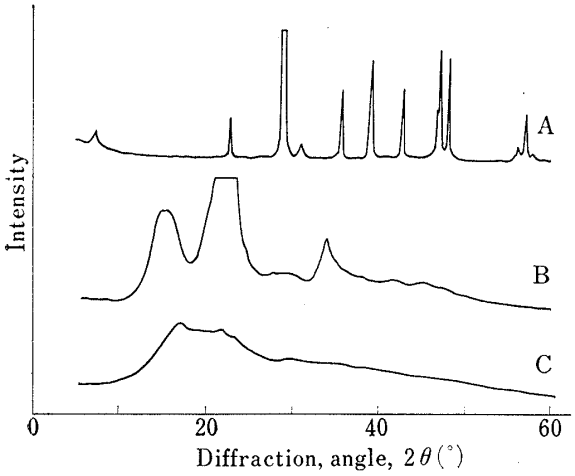

Fig. 3. X-Ray Diffraction Patterns of Materials tested as Diluents

A: $CaCO_3$, B: cellulose, C: dextrin.

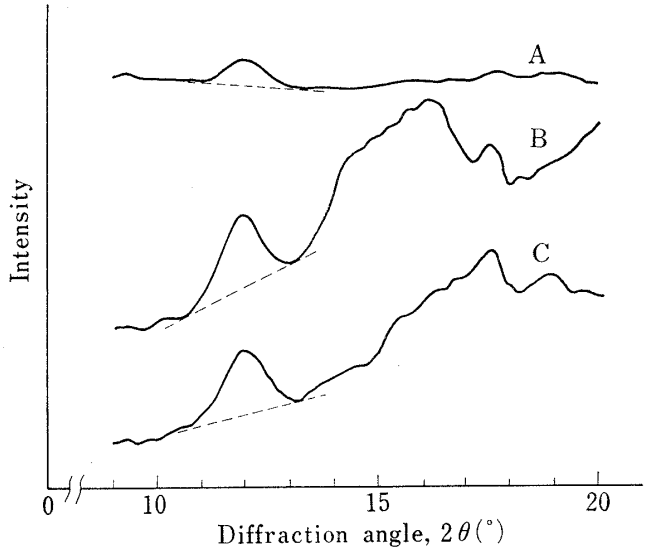

Fig. 4. X-Ray Diffraction patterns of Mixtures of Each Diluent, Inulin and α-Al_2O_3 (60: 40: 15)

A: $CaCO_3$+inulin+α-Al_2O_3, B: cellulose+inulin+α-Al_2O_3, C: dextrin+inulin+α-Al_2O_3.

On the other hand, since cellulose and dextrin consist of carbon, hydrogen and oxygen, with very small mass absorption coefficients, the effect of its addition on the intensity of the diffraction line of inulin should be slight. Cellulose has a strong peak near 15° in its X-ray diffraction pattern, so the slope of the base line at the peak of inulin becomes rather sharp. In the case of dextrin, the peak near 20° is broad, and the diffraction pattern is similar to that of the plant sample. For these reasons, dextrin was used as a diluent for the calibration curve.

Effect of Grinding

The sample has to be ground to a fine powder in order to obtain better precision; grinding reduces the number of strong diffraction spots from large particles and reduces the preferred orientation of crystalline particles, thus improving the reproducibility of measurement. However, grinding also broadens the diffraction line and lowers the intensity. This decrease of intensity on grinding is unavoidable in quantitative analysis because the sample and internal standard must be powdered and mixed by grinding. Therefore, the effect of grinding is an important factor governing the reproducibility of the intensity of diffraction lines.

Figure 5 shows the effect on the intensity of diffraction lines (12.0° of inulin and 57.4° of α-Al_2O_3) using various grinding methods. With a ball mill, the diffraction intensity of α-Al_2O_3 and especially that of inulin both decreased markedly with the grinding time because the grinding decreases the crystalline size and increases the lattice strain.

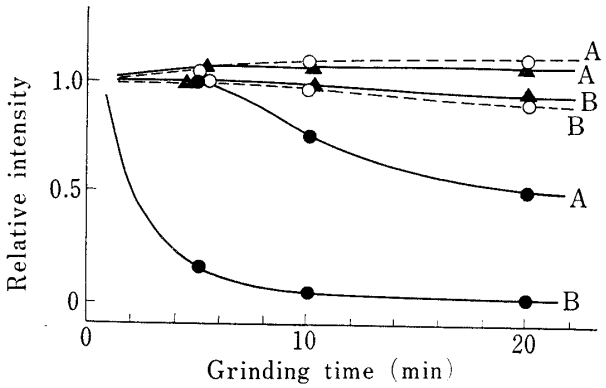

Fig. 5. Effect of Grinding on the Intensity of Diffracted X-Rays

A: diffraction line (57.4°) of α-Al_2O_3, B: diffraction line (12.0°) of inulin.
—●—: ball mill, ---○---: agate mortar,
—▲—: propeller-type mill.

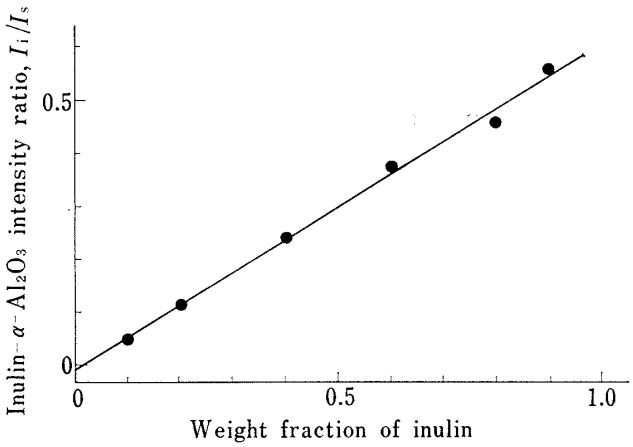

Fig. 6. Calibration Curve for Inulin Analysis Using α-Al_2O_3 as an Internal Standard

Procedures

The above results led to the following recommended procedure. The sample chips (*ca.* 1.5 g) are air-dried at room temperature, ground to a powder with a propeller-type mill, and kept in a desiccator for 1 week. One gram of the powdered sample is mixed with 0.15

Table I. Analytical Results for Inulin in the Underground Parts of Various Medicinal Plants

Materials	Inulin content (%)
Campanulaceae	
Adenophora triphylla var. *japonica* Hara[a]	10
Campanula punctata Lam.[b]	16
Codonopsis lanceolata Trautv.[b]	40
Platycodon grandiflorum A. DC.[c]	45
Compositae	
Adenocaulon himalaicum Edgew.[b]	21
Arctium lappa L.[d]	34
Aster tataricus L.[c]	32
Cirsium japonicum DC.[b]	16
Dahlia pinnata Cav.[c]	72
Ligularia fischeri Turcz.[b]	<4
Petasites japonicus Maxim.[b]	17
Syneilesis palmata Maxim.[b]	<4
Atractylodes japonica Koidz.[c]	13
A. lancea DC. var. *chinensis* Kitam. (Seihoku-sôjutsu)[d,e]	26
A. ovata A. P. DC. (Kara-byakujutsu)[d,e]	40
Inula helenium L.[c]	32
Saussurea lappa C.A. Clarke (Mokkô)[d,e]	40

a) Collected at Mt. Nijô.
b) Collected at Mt. Kongô.
c) Cultivated.
d) Obtained from Osaka market.
e) Crude drug sample.

g of α-Al_2O_3 in an agate mortar for 5 min. After keeping the mixture in a desiccator for 1 day, it is packed into the cavity of an aluminium specimen holder for diffractometric analysis.

The measurement for one sample takes less than 5 min.

Calibration Curve

The calibration curve for analysis of inulin in plants was prepared using α-Al_2O_3 as an internal standard and dextrin as a diluent (Fig. 6). The limit of detection for inulin was 4.0% and the standard deviation for a 60% sample was 3.9% for 10 consecutive measurements.

Analytical Results

Some medicinal plants (Campanulaceae and Compositae) were analyzed by the developed procedures, and the results are given in Table I. A relatively high inulin content was found in the cases of *Dahlia pinnata* Cav., *Platycodon grandiflorum* A. DC., *Codonopsis lanceolata* Trautv., *etc.*, which were thought to contain a large amount of inulin in their underground parts on the basis of microscopic examinations.

Conclusion

An X-ray diffractometric method for the determination of inulin in plants was developed, using α-Al_2O_3 as an internal standard and dextrin as a diluent. This procedure does not require complex pretreatment (only drying and grinding), and in addition, the time required for analysis is short. This method is suitable for analyses of inulin contents in various parts of plants involving fairly large numbers of samples.

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