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MOLECULAR CHARACTERIZATION OF SINISTRIN

E. NITSCH*,
Laevosan-Gesellschaft, Estermannstraße 17, A-4020 Linz (Austria)
W. IWANOV**, AND K. LEDERER
Institut für chemische und physikalische Technologie der Kunststoffe an der Montanuniversität Leoben, Franz-Josef-Straße 18, A-8700 Leoben (Austria)

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ABSTRACT

The molecular weight distribution of sinistrin (Inutest ®, Laevosan Ges., Linz, Austria), determined by analytical gel-permeation chromatography, using narrow fractions ($\overline{M}_w/\overline{M}_n < 1.07$) obtained by preparative gel-permeation chromatography, covered the range 800–16,000 with $\overline{M}_n = 2{,}500$ and $\overline{M}_w = 3{,}500$. From viscosity measurements on dilute, aqueous solutions, the relation $[\eta] = 0.28 \times M^{0.3}$ was obtained, indicating a branched molecular structure; the largest molecules can be described by a sphere with $r = 23$ Å. Comparison of the content of glucose and reducing sugars in the fractions with the molecular weight determined by vapour-pressure osmometry indicated that a glucose end-group is present in the majority of the molecules. The percentage of glucose end-groups is higher in the fractions of lower molecular weight. From this finding, speculations on the biosynthesis of sinistrin are made. The specific optical rotation of sinistrin fractions decreases linearly with $1/\overline{M}_n$.

INTRODUCTION

As early as 1879, Schmiedeberg[1] reported the isolation of a new carbohydrate from the bulbs of red squill (*Urginea maritima*), which he termed sinistrin. By cryoscopy, Schlubach and co-workers[2,3] determined the degree of polymerisation ($\overline{P}_n$) of sinistrin to be 15. Methylation studies[2,3] indicated sinistrin to be a β-D-fructan of the inulin type with branching at position 6. It is now generally accepted that the reducing fructose residue in inulin is masked by an α-D-glucopyranose end-group[4]. Such a feature has not yet been established for sinistrin.

Recently, sinistrin has been found to be very useful for the determination of renal clearance[5,6] in place of inulin and has been made commercially available for

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*To whom requests for reprints should be sent.

**Present address: Steyr-Daimler-Puch AG, Werk Steyr, A-4400 Steyr, Austria.

this purpose (Inutest ®). Like inulin[7], sinistrin is filtered in the kidneys through the glomeruli only and is neither reabsorbed nor secreted by the cells of the tubuli. Sinistrin is water-soluble at room temperature (in any concentration), an advantage in clinical use, whereas inulin is nearly insoluble in cold water and can only be used after heating suspensions of, for example, 0.1 g/ml up to 80°, in order to provide clear solutions. This requirement is not only inconvenient, but also may cause partial hydrolysis.

We now report on the fractionation of sinistrin by preparative gel-permeation chromatography, and on the characterisation of the fractions. The two main objectives of this study were the determination of the molecular-size distribution (of crucial importance for the function of sinistrin in the determination of renal clearance) and the identity of the reducing end-group.

MATERIALS AND METHODS

Reducing sugars were determined by using *p*-anisyl Tetrazolium Blue[8]. The results are given as oxidation values (O_{Fru}), *i.e.*, the apparent fructose content in g per 100 g of dry, unhydrolysed sample. D-Glucose was determined, after hydrolysis (0.01M HCl, 60 min, 100°), enzymically by the GOD–Perid method[9]. The results are expressed as g of D-glucose per 100 g of dry, unhydrolysed sample. Acetylation was carried out with acetic anhydride in pyridine[10].

Sinistrin. — The preparation[6] is that used to produce commercial sinistrin (Inutest ®). Dried bulbs (100 kg) of red squill (*Urginea maritima*, Schütte & Co., Hamburg) were cut to a maximum diameter of 4 mm and were then mixed with 30% aqueous methanol (200 kg). After 24 h at room temperature, the mixture was percolated by using the same solvent at 4 kg/h until 300 kg of extract were collected. The pH of the extract was brought to 9 with a concentrated slurry of calcium hydroxide (250 g) in water, and the extract was filtered, and concentrated *in vacuo* to give a thick syrup (75 kg) containing 80% of solid. Methanol (30 kg) was added with vigorous stirring to give an almost clear solution, which was cooled to 15°. A mixture of conc. hydrochloric acid (3 litres) and methanol (8 kg) was added with vigorous stirring followed by methanol (250 kg) to precipitate sinistrin as a very viscous, yellowish white material. After standing for a short time, the clear, yellow supernatant was decanted. The crude sinistrin (~40 kg) was triturated with methanol (3 × 40 kg) to give a fine powder (~35 kg) which was collected by centrifugation. A solution of this material in water (70 kg) was stirred with activated carbon (3 kg) and filtered, and the filtrate was passed at 15 litres/h over a mixed column of weak cation-exchange (H^+) resin (5 litres) and strong anion-exchange (HO^-) resin (10 litres).

The resulting aqueous solution of sinistrin was concentrated *in vacuo* to a thick syrup which was dried at 80° as a thin layer in steel pans.

Gel-permeation chromatography. — (*a*) *Preparative scale.* The procedure was carried out at room temperature by using three columns (200 × 80 mm), in series, of Sephadex G-50 fine (Pharmacia, Uppsala) and elution with 1-butanol-saturated water at 1650 ml/h; 50 ml of a 25% solution were fractionated in each run. From each run, 24 fractions (138 ml each) were collected, and the corresponding fractions from 20 runs were combined, concentrated *in vacuo* at 40° to 25%, and refractionated as described above. From this second fractionation, a peak fraction corresponding to 25% of the loaded sample introduced was collected. The fractions thus obtained were then concentrated to ~10% and desalted by using Lewatit MP 600 (HO^-) resin and IMAC C16P (H^+) resin. The eluates were further concentrated and finally dried over P_2O_5 at 80°/8 Torr.

(*b*) *Analytical scale.* This procedure was carried out at 50° on two columns (1000 × 6 mm), in series, of controlled-pore glass (CPG 10, pore size 75 Å, 200–400 mesh; Electro Nucleonics Inc., Fairfield, N.J.) by elution with sodium phosphate buffer (0.02M, pH 6.8) containing sodium azide (0.02 g/100 ml); 100 μl (0.2 g/100 ml) was used for each separation. The flow rate was 25 ml/h, and the effluent was monitored by a differential refractometer (Type 51.00; Knauer, Berlin).

Physical methods. — Vapour-pressure osmometry was carried out in the usual way[11] with a Knauer (Berlin) instrument, using the special temperature probe for aqueous solutions. Measurements on sinistrin and its fractions were performed on 1–5% aqueous solutions at 50°, with use of aqueous solutions of sucrose for calibration. Benzene was the solvent for acetylated samples at 50°, and benzil for calibration.

Viscosity measurements on dilute solutions (0.03–0.12 g/ml) of sinistrin and its fractions in distilled water at 25° were carried out in the usual way[12] with a Mikro-Ubbelohde Viscometer (Schott, Mainz) which required only 2 ml of solution. The average rate of shear for water at 25° is ~2500 sec^{-1} in this instrument.

Specific optical rotations were determined at 589 and 578 nm with a Perkin–Elmer Model 141 polarimeter and aqueous solutions ($c = 0.02$ g/ml) at 20°.

RESULTS

The result of preparative gel-permeation chromatography of sinistrin is shown in Fig. 1 in comparison with that for inulin. The peak for sinistrin is only slightly broader than for inulin, indicating a very similar distribution of molecular sizes. The oxidation value, glucose content, and specific rotation of sinistrin and the fractions obtained by preparative gel-permeation chromatography are given in Table I. These materials and their acetylated derivatives were also characterised by vapour-pressure osmometry. The results given in Table II* can be compared with the values of the number-average molecular weight calculated from end-group determination. If each sinistrin molecule carries either a glucose or a reducing fructose end-group, the number (N) of molecules (in mol) in 1 g of sample and the corresponding value of the number-average molecular weight ($M_{n,end}$) can be calculated from the values

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*The different values of $M_{n,vo}$ for fractions 5 and 9 were determined in two independent measurements.

for O_{Fru} and [Glc] in Table I according to equation *1*, where $M_{Fru} = M_{Glc} = 180.16$ g/mol (molecular weight of fructose and glucose), and $M_{n,end} = 1/N$.

$$N = \frac{O_{Fru}}{M_{Fru} \times 100} + \frac{[Glc]}{M_{Glc} \times 100} \tag{1}$$

Fig. 2 shows typical eluograms determined by analytical gel-permeation chromatography. The peak maxima are designated as peak elution volumes (V_e). The values of V_e for sinistrin and sinistrin fractions 3–21 are given in Table III.

Using the data in Tables II and III, calibration of the analytical system can be accomplished (Fig. 3), and the eluogram of sinistrin in Fig. 2 can then be transformed into the differential mass distribution of molecular weight shown (Fig. 4)[13]. The peak broadening due to axial dispersion can be neglected, because of the comparatively large width of the peak observed with (unfractionated) sinistrin. In Fig. 4, the scale of the ordinate was chosen to satisfy the normalizing condition:

$$\int_{\log M=0}^{\log M=\infty} \frac{dI}{d\log M} \cdot d\log M = 1 \tag{2}$$

with the consequence that

$$\int_{\log M=\log M_1}^{\log M=\log M_2} \frac{dI}{d\log M} \cdot d\log M = w_{M_1,M_2}, \tag{3}$$

where w_{M_1,M_2} = the portion of the total mass of the sample having a molecular weight between M_1 and M_2.

TABLE I

ANALYTICAL DATA FOR SINISTRIN AND ITS FRACTIONS

	O_{Fru}[a]	[Glc][b]	$[\alpha]^{20}_{589}$ (degrees)	$[\alpha]^{20}_{578}$ (degrees)
Sinistrin	1.44	4.06	−39.4	−43.0
Fraction 1	0.74	3.26	−39.8	−42.7
2	0.89	0.95	−46.1	−47.5
3	0.93	1.13	−44.6	−47.1
5	0.29	1.65	−44.4	−45.7
7	0.78	1.92	−42.1	−43.3
8	0.32	2.25	−43.8	−44.6
9	0.55	2.58	−41.5	−45.1
11	0.51	3.45	−40.2	−42.1
13	0.64	4.45	−39.1	−41.0
15	0.76	5.71	−34.7	−37.3
18	1.11	7.18	−32.9	−36.0
19	1.35	8.18	−31.2	−32.0
20	1.65	8.59	−30.2	−31.9
21	2.36	10.21	−27.3	−29.7

[a] Oxidation value, *i.e.*, the apparent fructose content in g per 100 g of dry, unhydrolysed sample.
[b] Content of D-glucose in g per 100 g of dry, unhydrolysed sample.

TABLE II

MOLECULAR WEIGHT DATA FOR SINISTRIN AND ITS FRACTIONS

	$\overline{M}_{n,VO}$[a]	$\overline{M}_{n,ac,VO}$[b]	$\overline{M}_{n,acet}$[c]	$\overline{M}_{n,end}$[d]
Sinistrin	3057	5270	2950	3280
Fraction 1	—	—	—	4500
2	—	—	—	9800
3	14160	15500	8700	8750
5	10100,12100	11700	6550	9290
7	8500	11200	6260	6680
8	—	—	—	7010
9	8630,8090	8990	5020	5760
11	5092	6840	3800	4556
13	4084	5360	3000	3540
15	3429	4810	2670	2780
18	2443	3910	2180	2170
19	—	—	—	1890
20	—	—	—	1760
21	1528	2650	1450	1430

[a] Determined by vapour-phase osmometry in aqueous solution. [b] Determined, for acetylated samples, by vapour-phase osmometry in benzene solution. [c] Calculated from $M_{n,ac,VO}$ by assuming that acetylation leads to complete esterification of free hydroxyl groups without any other change of molecular structure. [d] Calculated from the values for O_{Fru} and [Glc] in Table I, according to equation *1*.

TABLE III

VISCOSITY, ELUTION VOLUME (V_e), AND PEAK-BROADENING (σ) DATA FOR SINISTRIN AND ITS FRACTIONS

	$[\eta]$ *(ml/g)*	V_e *(ml)*	$\overline{M}_w/\overline{M}_n$	σ *(ml)*
Sinistrin	3.35	37.8	1.40	—
Fraction 1	—	—	—	—
2	—	—	—	—
3	3.95	32.1	1.07	1.48
5	3.76	33.4	1.07	1.54
7	3.58	34.6	1.07	1.52
8	—	35.0	1.07	1.54
9	3.85, 3.70	35.75	1.06	1.50
11	3.02	36.7	1.04	1.41
13	2.7, 3.57	37.6	1.04	1.41
15	3.0, 3.25	38.2	1.04	1.42
18	2.66	39.5	1.06	1.56
19	—	39.9	1.06	1.61
20	—	40.1	1.05	1.56
21	2.50, 2.40	40.7	1.04	1.47

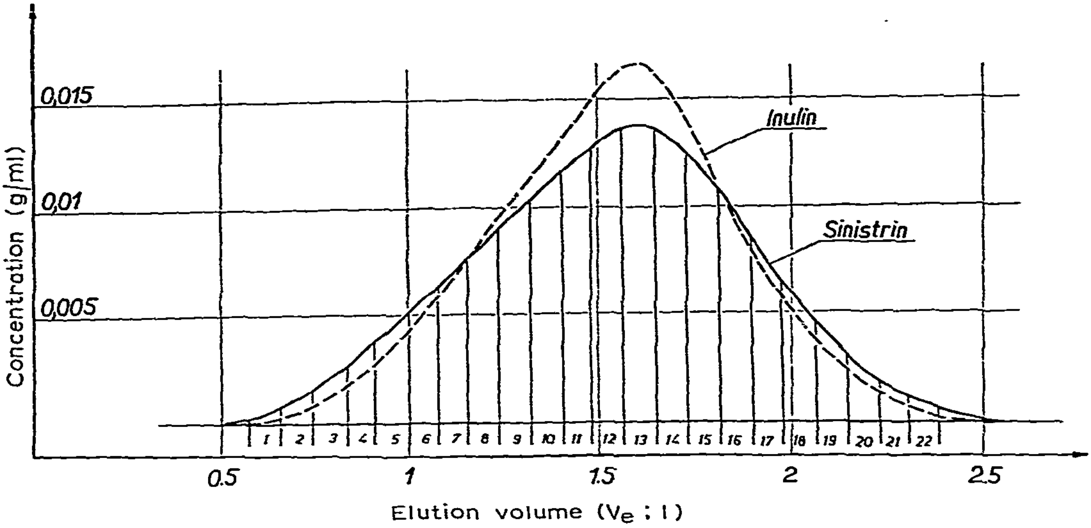


Fig. 1. Eluogram obtained by preparative gel-permeation chromatography of sinistrin and inulin; the numbers 1–22 indicate the sinistrin fractions.

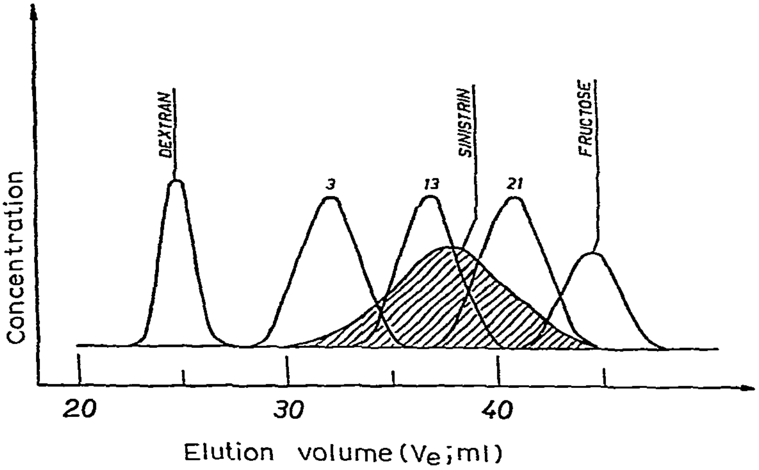


Fig. 2. Eluogram obtained by analytical gel-permeation chromatography for dextran, sinistrin, fructose, and sinistrin fractions 3, 13, and 21.

The integrals given above can easily be determined from the respective areas under the curve shown in Fig. 4.

In Table III, the peak broadening (σ) observed in analytical gel-permeation chromatography of sinistrin fractions is also given; σ is equal to the standard deviation of the Gaussian that provides the best fit to the peak observed. As can be seen from Fig. 2, such a monodisperse sample as fructose shows a peak broadening due to axial dispersion[14] of the same order of magnitude as that observed with sinistrin fractions.

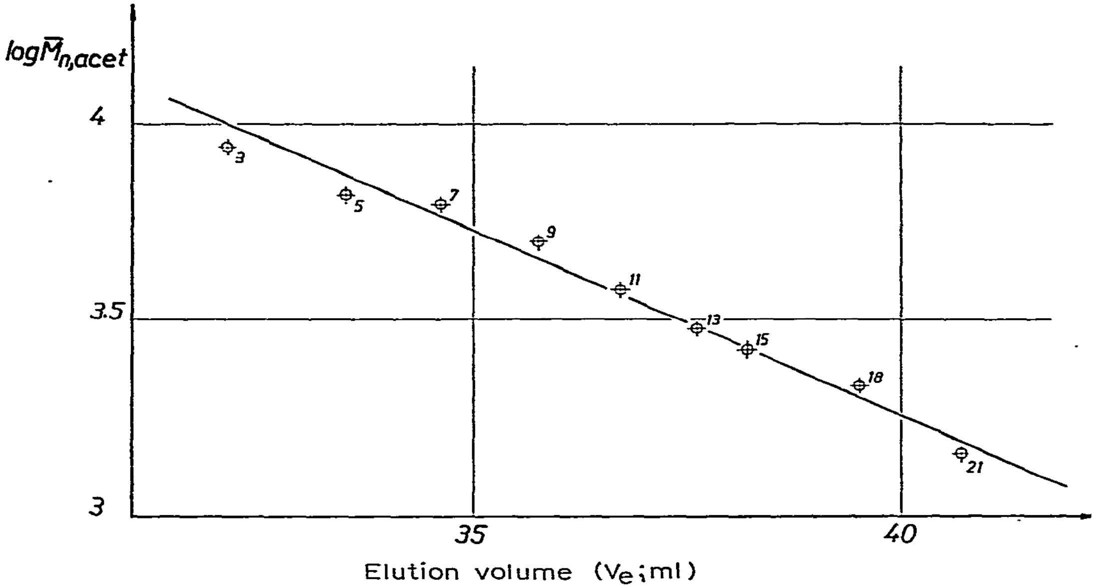


Fig. 3. Calibration curve for analytical gel-permeation chromatography obtained with fractions 3–21 of sinistrin (see Tables II and III).

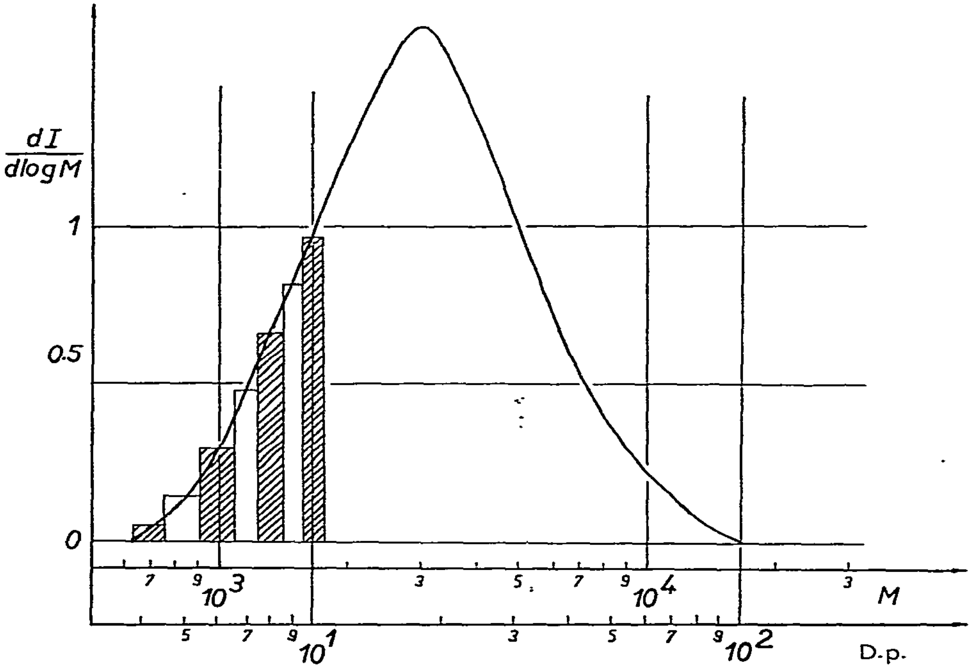


Fig. 4. Differential mass distribution of molecular weight (M) for sinistrin in the normalized (*cf.* text) plot dI/dlog M *vs.* log M.

Whereas for the broad peak observed in the eluogram of (unfractionated) sinistrin (see Fig. 2), the peak broadening due to axial dispersion can be neglected when deriving the molecular weight distribution, the narrow peaks in the eluogram for sinistrin fractions must be corrected for axial dispersion to estimate their heterogeneity. In the present case, it can be assumed that the number of theoretical plates, $N = (V_e/\sigma)^2$, is approximately the same for all the sinistrin fractions studied, because the value of N for fructose ($N_{Fru} = 1200$), which has a low molecular weight, did not differ considerably from the value for dextran ($N_{Dex} = 1000$), which has a very high molecular weight. The peak broadening that would be observed without axial dispersion could therefore be estimated by the following equation:

$$\sigma^2_{corr} = \sigma^2_{obs} - \frac{V_e^2}{N_{Fru}}, \qquad (4)$$

where σ_{obs} = observed peak broadening, σ_{corr} = peak broadening corrected for axial dispersion, V_e = peak elution volume of the peak considered, and N_{Fru} = the number of theoretical plates determined with fructose ($N_{Fru} = 1200$ in the present case).

From the values of σ_{corr} calculated for the various sinistrin fractions, the non-uniformity parameter $\overline{M}_w/\overline{M}_n$ can be estimated by the following equation[15]:

$$\overline{M}_w/\overline{M}_n = e^{-(2.303 \times B \times \sigma_{corr})^2}, \qquad (5)$$

where $\overline{M}_w$ = weight-average molecular weight of the fraction, $\overline{M}_n$ = number-average molecular weight of the fraction, and B = the slope of the calibration curve given in Fig. 3 at the molecular weight $\overline{M}_n$ of the fraction.

Equation *5* is strictly valid only for samples having a narrow distribution of molecular weight ($\overline{M}_w/\overline{M}_n \leqslant 1.10$). Therefore, equation *5* is not applicable for an estimate of the inhomogeneity of (unfractionated) sinistrin. Another prerequisite for the validity of equation *5* is that the molecular weight calibration may be described by an equation of the following form:

$$\log M = A - B \times V_e, \qquad (6)$$

which is fulfilled by the straight line shown in Fig. 3 with $A = 7.05$ and $B = 0.095$ ml^{-1}.

The values of $\overline{M}_w/\overline{M}_n$ for sinistrin fractions estimated in this way are also summarized in Table III. The value for $\overline{M}_w/\overline{M}_n$ of sinistrin, also given in Table III, was determined from the plot in Fig. 4 by integration according to:

$$\overline{M}_w = \int_{\log M=0}^{\log M=\infty} \left(\frac{dI}{d\log M}\right) \cdot M \cdot d\log M \qquad (7)$$

and

$$\overline{M}_n = 1 \Bigg/ \left[\int_{\log M=0}^{\log M=\infty} \left(\frac{dI}{d\log M}\right) \cdot \frac{1}{M} \cdot d\log M\right], \qquad (8)$$

leading to $\overline{M}_n = 2505$ and $\overline{M}_w = 3495$.

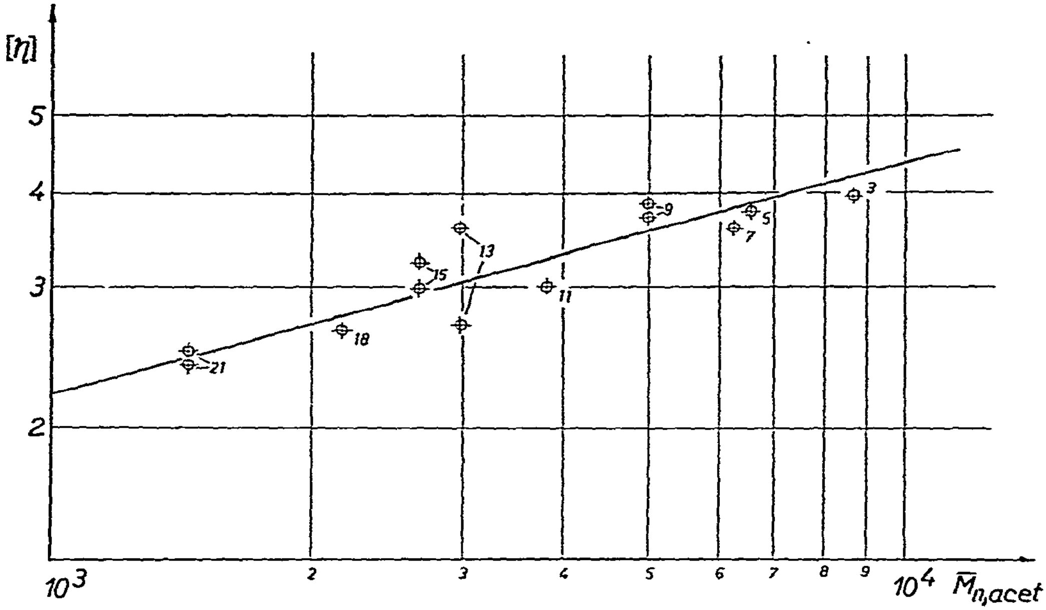


Fig. 5. Intrinsic viscosity–molecular weight relationship for sinistrin and fractions 3–21 in the plot log [η] *vs.* log $M_{n,acet}$: [η] measured in water at 25°.

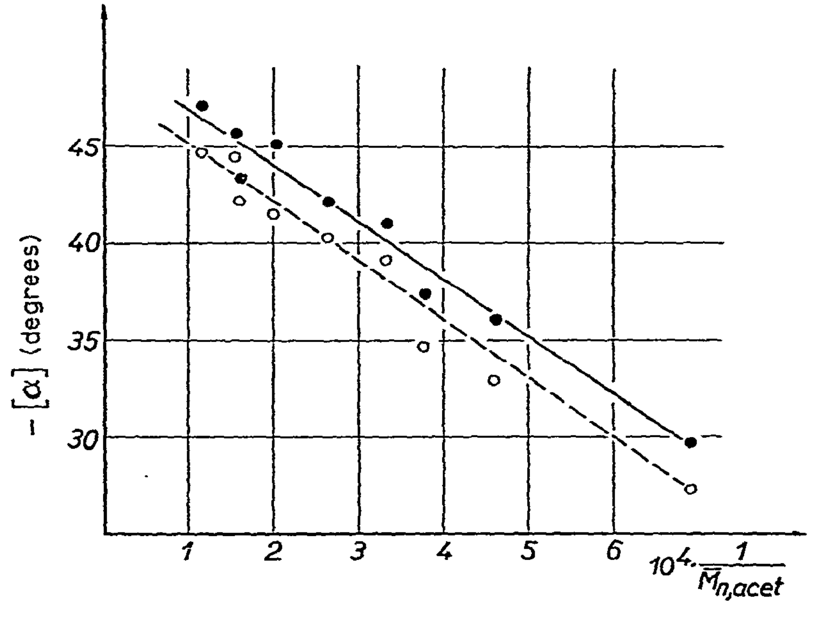


Fig. 6. Specific optical rotation–molecular weight relationship for sinistrin fractions: [α] measured in dilute, aqueous solutions (c = 0.02 g/ml) at 20°; ○, λ = 589 nm; ●, λ = 578 nm.

The values of the intrinsic viscosity of sinistrin and sinistrin fractions are also given in Table III. They were determined as described above. The different values obtained with fractions 9, 13, 15, and 21 were measured in independent experiments. With each sample, an exceptionally high value of the Huggins coefficient (k′) in the range 0.7–1.8 was found. The Huggins coefficient describes the concentration dependence of the reduced viscosity according[16] to equation *9*.

$$\eta_{red} = [\eta] + k'[\eta]^2 c \qquad (9)$$

The dependence of the intrinsic viscosity on the molecular weight, $M_{n,acet}$ (*cf.* Table II) is given in Fig. 5 as a double logarithmic plot. The straight line drawn in this plot is given analytically by equation *10*.

$$[\eta] = 0.275 \times M^{0.30} \qquad (10)$$

DISCUSSION

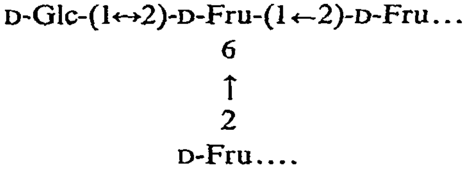
The values of the specific optical rotation, $[\alpha]$, shown in Table I are in good correlation with the usual finding[17] that, in a polymer homologous series, $[\alpha]$ changes linearly with $1/\overline{M}_n$, as is shown in Fig. 6.

The evaluation of the eluogram of sinistrin (Fig. 2), obtained by analytical gel-permeation chromatography, in terms of absolute molecular weight is facilitated by the fact that fractions having a very narrow distribution of molecular weight ($M_w/M_n < 1.07$, see Table III) were available. However, the interpretation was complicated by the lack of close agreement of corresponding values for the molecular weight of sinistrin fractions determined in different ways (see Table II). Discrepancies are mainly given between the values of $\overline{M}_{n,VO}$ and $\overline{M}_{n,acet}$. There are two reasons to prefer the values of $\overline{M}_{n,acet}$ in the further evaluation of the data. First, the extraordinarily high values of the Huggins coefficient found in the viscosity measurements reported above strongly indicate that molecular aggregation takes place in aqueous solution, which would explain why the measured values of $\overline{M}_{n,VO}$ are always considerably greater then the respective values of $\overline{M}_{n,acet}$. Second, the applied acetylation procedure led to complete esterification (proved by infrared spectroscopy), and there is no reason to assume that this procedure causes changes, other than esterification, in a polyfructosan.

The differential mass distribution of molecular weight thus obtained shows that the largest molecules of sinistrin have a molecular weight $M_{max} = 16{,}000$, whereas the smallest molecules appear to be the pentamer. It should be stressed, at this point, that the curve in Fig. 4 is not a continuous function but a histogram. The non-continuous character must especially be kept in mind at small values of $\overline{M}_n$, as indicated in Fig. 4.

Taking into account the limited precision of vapour-pressure osmometry, there is a rather good agreement between the values of $\overline{M}_{n,acet}$ and $\overline{M}_{n,end}$ (Table II), indicating that the assumption that each sinistrin molecule carries at one end either a glucose or a reducing fructose end-group may indeed be true. Evaluation of the data for O_{Fru} and [Glc] given in Table I by equation *1* leads to the conclusion that 74% of all sinistrin molecules carry a glucose end-group and 26% a reducing fructose end-group. By the same argument, the lowest percentage of sinistrin molecules having a glucose end-group is suggested for fraction 2 (52%), whereas the highest such percentage is suggested for fraction 15 (88%). If random hydrolysis of sinistrin molecules occurred during the preparation procedure, the percentage of molecules having a glucose end-group in a certain fraction should be lower when the respective molecular weight is lower.

It seems to be reasonable to assume that the biosynthesis of sinistrin starts by transfructosidation of sucrose[18], and normally leads to the following structure:



$$\begin{array}{ll} \text{D-Glc-}(1\leftrightarrow 2)\text{-} & \text{D-Fru-}(1\leftarrow 2)\text{-D-Fru}\ldots \\ & \quad 6 \\ & \quad \uparrow \\ & \quad 2 \\ & \text{D-Fru}\ldots. \end{array}$$

To explain the finding of the dependence of the percentage of molecules having glucose end-groups on molecular weight, one can speculate that enzymic hydrolysis at the sucrose end-group, by reversing the first step of biosynthesis, might be a side-reaction that competes with the growth of sinistrin molecules by addition of fructose sub-units. Such a competing reaction would occur with a probability that is higher when the time necessary to build up the sinistrin molecule is longer, *i.e.*, the higher the molecular weight. The result of the viscosity measurements shown in Fig. 5 can be considered as further evidence for a branched structure of sinistrin, since the value of the exponent[19] in equation *10*, $a = 0.30$, is much smaller than 0.5. From the result expressed by equation *10*, an estimate of the molecular dimension of the largest sinistrin molecules ($M_{max} = 16,000$) can be made. For estimates of hydrodynamic properties, it is appropriate to approximate the shape of a branched macromolecule to a sphere with equivalent density ζ_e. According to general hydrodynamic theory, we can then formulate[19]:

$$[\eta] = 2.5/\zeta_e, \tag{11}$$

with $\zeta_e = M(v_e.A)$, where M = molecular weight, v_e = volume of the equivalent sphere (= sphere with the same properties in viscosity measurements), and A = Avogadro's number.

We can calculate from equations *10* and *11* that the radius of a sphere that is hydrodynamically equivalent to the largest sinistrin molecule ($M_{max} = 16,000$) is ~23 Å. This value compares very well with recent estimates of the size of the inulin molecule by Middleton[20] and strongly supports conclusions concerning the glomerular pore size presented by the same author.

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REFERENCES

1 O. SCHMIEDEBERG, *Hoppe-Seyler's Z. Physiol. Chem.*, 3 (1879) 112–133.
2 H. H. SCHLUBACH AND W. LOOP, *Justus Liebigs Ann. Chem.*, 523 (1936) 130–143.
3 H. H. SCHLUBACH AND O. K. SINH, *Justus Liebigs Ann. Chem.*, 544 (1940) 11–116.
4 K. HOLZER, H. WITTMANN-ZINKE, AND A. ZINKE, *Monatsh. Chem.*, 88 (1957) 11–24.
5 B. WATSCHINGER AND I. KOBINGER, *Wien. Z. Inn. Med.*, 45 (1964) 219–228.
6 Austrian Pat., 304,769 (1973).
7 J. A. SHANNON AND H. W. SMITH, *J. Clin. Invest.*, 14 (1935) 393–401.
8 N. D. CHERONIS AND M. C. ZYMANIS, *Mikrochim. Acta*, (1957) 769–783.
9 W. WERNER, H.-G. REY, AND H. WIELINGER, *Z. Anal. Chem.*, 252 (1970) 224–228.
10 W. N. HAWORTH AND H. R. L. STREIGHT, *Helv. Chim. Acta*, 15 (1932) 609–615.
11 C. A. GLOVER, in P. E. SLADE (Ed.), *Polymer Molecular Weights*, Part I, Dekker, New York, 1975, pp. 79–154.
12 J. SCHURZ, *Viskositätsmessungen an Hochpolymeren*, Berliner Union-Kohlhammer, Stuttgart, 1972, p. 36 ff.
13 L. H. TUNG AND J. C. MOORE, in L. H. TUNG (Ed.), *Fractionation of Synthetic Polymers*, Dekker, New York, 1977, p. 614 ff.
14 Ref. 13, p. 591 ff.
15 K. C. BERGER AND G. V. SCHULZ, *Makromol. Chem.*, 136 (1970) 221–229.
16 M. L. HUGGINS, *Physical Chemistry of High Polymers*, Wiley, New York, 1958, p. 86 ff.
17 H. H. SCHLUBACH AND F. LEDERER, *Justus Liebigs Ann. Chem.*, 635 (1960) 154–165.
18 K. JEREMIAS, *Naturwiss. Rundsch.*, 9 (1965) 362–363.
19 J. SCHURZ, *Struktur-Rheologie*, Berliner Union-Kohlhammer, Stuttgart, 1974.
20 E. MIDDLETON, *J. Membr. Biol.*, 34 (1977) 93–101.