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THE POLYSACCHARIDE GUM EXUDATE OF *PRUNUS AVIUM* VAR. *ACTIANA**

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Abstract—Analyses of the gum exudate from *Prunus avium* L. var. *actiana* (L.) Schneider have been compared with earlier results on the gum exudates from four other varieties of *P. avium* L. There are considerable chemical differences, which fit in with the known taxonomic variation within the species.

INTRODUCTION

NUMEROUS investigations of the gum exudates from different "cherry trees" have appeared in the literature. The cherry gum exudates which have been studied originate from a number of distinct species in the genus *Prunus*; gums from different varieties of one of these species have also been studied. The two most common types of cherry tree, the sour cherry (*P. cerasus* L.) and the sweet cherry (*P. avium* L.), are placed in Section *Cerasus* of the genus.[1] Much work has been carried out on the gum exudate of *P. cerasus* L., and its structure has been well characterized.[2-6] Gum exudates from different varieties of *P. avium* L. have been investigated; the Bratislava School have reported results from studies of gum exudates from three varieties of this species, var. *juliana* L.,[7] subsp. *avium*,[8] and var. *duracina* L.[9] A little work has been reported on the gum exudate from the Black Republican cherry tree[10] which is a variety of *P. avium* L.[11] The other types of "cherry tree", the gum exudates of which have been studied chemically, the wild cherry (*P. virginiana* L.),[12] and the cherry laurel (*P. laurocerasus* L.),[13] are taxonomically distinct from *P. cerasus* L. and *P. avium* L., being placed in Section *Padus* of the genus.

In 1966, Rosik and co-workers discovered large differences between the properties of the gum exudates from *P. cerasus* and two varieties of *P. avium* (i.e. var. *juliana* and subsp. *avium*); e.g. the specific rotation of *P. cerasus* gum was $-13{\cdot}6°$ compared with values of

* Part I in a projected series entitled "Gum Exudates of the Genus *Prunus*."

[1] K. MEYER, *Kultur geschichtliche und systematische Beiträge zur Gattung Prunus* (*Repertorium specierum novarum regui vegetablis*, Vol. 22), Dahlem, Berlin (1923).
[2] J. K. N. JONES, *J. Chem. Soc.* 558 (1939).
[3] J. K. N. JONES, *J. Chem. Soc.* 1055 (1947).
[4] J. K. N. JONES, *J. Chem. Soc.* 3141 (1949).
[5] P. ANDREWS, D. H. BALL and J. K. N. JONES, *J. Chem. Soc.* 4090 (1953).
[6] J. ROSIK, V. ZITKO and J. KUBALA, *Coll. Czech. Chem. Commun.* **31**, 1569 (1966).
[7] J. ROSIK, S. BAUER and J. KUBALA, *Coll. Czech. Chem. Commun.* **31**, 1072 (1966).
[8] J. ROSIK, V. ZITKO, S. BAUER and J. KUBALA, *Coll. Czech. Chem. Commun.* **31**, 3353 (1966).
[9] J. ROSIK, V. D. SCERBUCHIN and J. KUBALA, *Chem. Zvesti* **22**, 248 (1968).
[10] F. SMITH and R. MONTGOMERY, *The Chemistry of Plant Gums and Mucilages*, p. 269, Reinhold, New York (1959).
[11] N. H. GRUBB, *Cherries*, Crosby Lockwood, London (1949).
[12] C. L. BUTLER and L. H. CRETCHER, *J. Am. Chem. Soc.* **53**, 4160 (1931).
[13] J. B. PRIDHAM and H. G. T. WORTH, *Chem. Ind. (London)* 1403 (1968).

+55° and +48° for the varieties of *P. avium*[6–8]. Such large differences in properties between the gum exudates of such closely related species was not entirely unexpected, since the two species are recognized as being taxonomically distinct. The situation was made more complex in 1968 when Rosik and his colleagues found that the gum exudate from *P. avium* L. var. *duracina* L. showed great differences from those of the two varieties previously studied; e.g. var. *duracina* gum had a negative specific rotation (−19°) and contained a considerable amount of 4-*O*-methyl-D-glucuronic acid, while the gums of var. *juliana* and subsp. *avium* had positive specific rotations and contained at most traces of 4-*O*-methyl-D-glucuronic acid. The gum exudate of *P. avium* L. *actiana* (L.) Schneider was therefore examined in order to investigate further the chemical taxonomy of the *P. avium* complex.

RESULTS

After purification, the gum polysaccharide from this tree was shown to have the properties listed in Table 1. Samples of the gum were examined by zone electrophoresis on cellulose acetate film, by ion-exchange chromatography on DEAE-cellulose,[14] by molecular-sieve chromatography on "Bio-Gel P-300",[15] and "Sepharose-4B",[16] and by ultracentrifugation. Since no sharp discontinuities in the properties of the molecular species were indicated, it seems probable that *Prunus avium* var. *actiana* gum contains a continuous spectrum of related molecular species. The gums from *P. avium* L. var. *juliana*, var. *duracina*, and subsp. *avium* have been shown to be homogeneous on ultracentrifugation.[7–9]

TABLE 1. ANALYTICAL DATA FOR GUM EXUDATES FROM DIFFERENT VARIETIES OF *P. avium*

	Var. *actiana* (this paper)	Subsp. *avium* (Ref. 8)	Var. *juliana* (Ref. 9)	Var. *duracina* (Ref. 9)	Cv. Black Republican (Ref. 10)
Nitrogen, %	0·15	n.d.	n.d.	n.d.	n.d.
Protein, %	0·93	n.d.	n.d.	n.d.	n.d.
Intrinsic viscosity	161	n.d.	n.d.	n.d.	n.d.
Methoxyl content,	0·04	n.d.	0·00	0·77	0·00
$[\alpha]_D$	−3°	+48°	+55°	−19°	−9°
Equivalent weight	1496	1990	1760	1790	n.d.
∴ uronic acid, %	11·7	8·8	10·0	9·8	n.d.
Uronic acid anhydride, %	13·5	n.d.	n.d.	n.d.	n.d.
Formic acid release on periodate oxidation (mole/equivalent)	2·36	3·00	3·25	2·35	n.d.
Periodate reduced (mole/equivalent)	7·10	6·20	8·50	6·4	n.d.
Periodate reduced / Formic acid released	3·01	2·06	2·61	2·72	n.d.
L-Arabinose, %	51	47	51	53	present
D-Galactose, %	25	37	28	27	present
D-Xylose, %	4	6	7	5	present
D-Mannose, %	4	1	4	5	present
L-Rhamnose, %	4	trace	trace	trace	trace
D-Glucuronic acid, %	12	9	10	5	present
4-*O*-Methyl-D-glucuronic acid, %	—	trace	trace	5	—
Mw ($\times 10^{-6}$)	1·750	0·431	0·250	0·156	—

[14] M. A. JERMYN, *Australian J. Biol. Chem.* **15**, 789 (1963).
[15] D. M. W. ANDERSON, I. C. M. DEA, S. RAHMAN and J. F. STODDART, *Chem. Commun.* 145 (1965).
[16] D. M. W. ANDERSON, I. C. M. DEA and A. C. MUNRO, *Carbohyd. Res.* **9**, 363 (1969).

P. avium var. *actiana* gum was shown to be comprised of D-galactose, D-mannose, D-xylose, L-rhamnose, L-arabinose, and D-glucuronic acid. On total acid hydrolysis no 4-*O*-methyl-D-glucuronic acid was detected, and on partial acid hydrolysis no 4-*O*-methyl-D-glucuronic acid-containing aldobiouronic acids were detected; the methoxyl content of 0·04 per cent is in agreement with these findings. A study of the data presented in Table 1 shows some quite striking differences in the properties of the five varieties of *P. avium* L. gum studied to date. Quite large variations in sugar content of the gums are apparent. Thus var. *duracina* gum contains equal amounts of glucuronic acid and the 4-*O*-methyl analogue, while the gums from the other varieties contain at the most traces of the *O*-methyl sugar; var. *actiana* gum contains 4 per cent rhamnose while the gums from the other varieties contain only traces of this sugar; the mannose content varies from 1 per cent to 5 per cent, and the galactose content varies from 25 per cent to 38 per cent. Possibly the most striking variations are in specific rotation. Three of the gums (those of var. *actiana*, var. *duracina*, and the Black Republican cherry) have negative specification rotations, while the other two gums (those of var. *juliana* and subsp. *avium*) have quite strongly positive specific rotations. Such wide differences in specific rotations would seem to indicate some significant difference(s) in the molecular structure of the gums. It has been shown[17] that *Acacia* gums from species in Series Gummiferae with positive specific rotation, and the gums from species in Series Vulgares with negative specific rotation differ in their galactan framework, the arabinose side-chains, and the method of attachment of uronic acid residues to the galactan framework. It is perhaps significant that those *P. avium* gums with positive specific rotation have a lower ratio of periodate consumption to formic acid release than those with negative specific rotation.

This comparison of the gum exudates from five varieties of *P. avium* clearly indicates wide chemical differences within the species complex. This is good support for the taxonomic view that the *P. avium* complex is very varied. The differences in composition of the gums from the different varieties of *P. avium* are much greater than those found between the gums from different varieties of a species in the genus *Acacia* and are almost as large as the differences found between gums from species in different sections of this genus.[17]

EXPERIMENTAL

Specific rotations were determined at 589 nm using a Perkin–Elmer 141 Polarimeter at 23°. Rotary evaporations were carried out at or below 40°. Paper chromatography was carried out on Whatman Nos. 1 and 3MM paper with (*a*) benzene–butan-1-ol–pyridine–water (1:5:3:3, upper layer), (*b*) ethyl acetate–pyridine–water (10:4:3), (*c*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), and (*d*) butan-1-ol–ethanol–0·1 M HCl (1:10:5). Whatman No. 1 papers which had been pretreated with 0·5 M NaH_2PO_4 and air dried were used with solvent (*d*). The ratio of neutral sugars in the polysaccharide was determined colorimetrically[18] after elution from thick papers. The equivalent weight of the polysaccharide was determined by titration of a solution of electrodialysed gum with 0·01 M NaOH to a phenolphthalein end point; this led to an estimate of the uronic acid content of the polysaccharide. The uronic acid content and methoxyl content were determined by gas-phase i.r. methods.[19,20] The N content was determined by a semi-micro Kjeldahl method. Periodate oxidation of the gum was carried out at room temp in an unbuffered soln. The periodate consumption of the polysaccharide was estimated by back titration, and the formic acid released was estimated titrimetrically; constant values were obtained after 48 hr.[21] Intrinsic viscosity measurements were carried out in M NaCl using a suspended-level dilution viscometer at 25°. Light-scattering measurements were carried out using a

[17] D. M. W. Anderson and I. C. M. Dea, *Phytochem.* **8**, 167 (1969).
[18] M. Dubois, K. A. Gilles, J. R. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.* **28**, 350 (1956).
[19] D. M. W. Anderson and J. L. Duncan, *Talanta* **8**, 1 (1961).
[20] D. M. W. Anderson, S. Garbutt and S. S. H. Zaidi, *Anal. Chim. Acta* **29**, 39 (1963).
[21] R. Belcher, G. Dryhurst and A. M. G. Macdonald, *J. Chem. Soc.* 220 (1965).

SOFICA Photo Diffusometer, using the methods outlined in the literature.[22,23] Determinations were carried out at three different concentrations; the value of $\bar{M}w$ quoted is the average of these determinations.

Origin of Specimen

Nodules of the gum from *Prunus avium* L. var. *actiana* (L.) Schneider were collected by the author from a tree (botanically authenticated by Dr. J. Anliker, Federal Research Station, Wädenswil, Switzerland) in the Reinach suburb of Basle, Switzerland, in July 1968. Voucher specimens of the tree were collected by Herr P. Aellen, and are housed in the Herbarium, Royal Botanic Garden, Edinburgh, Scotland.

Purification of P. avium *var.* actiana *Gum*

The gum (95 g) was dissolved in water (4 l.), filtered, and dialysed. The polysaccharide was isolated as the freeze-dried product (87 g). A portion of this product (1 g) was dissolved in water, exhaustively electrodialysed, and freeze-dried to yield the gum acid. The analytical data for the gum are shown in Table 1. The methoxyl content of the gum is very low; no *O*-methyl sugars were detected in the gum hydrolysates.

The gum migrated as a single band on electrophoresis on strips (18 × 5 cm) of cellulose acetate film in both 0·1 M $(NH_4)_2CO_3$ buffer (pH 8·9) and 0·1 M acetate buffer (pH 4·7) at field strengths between 15 and 20 V/cm for 2–4 hr. Polysaccharide bands were visualized by a modification of the periodate-rosaniline hydrochloride method.[24] Ultracentrifugation of a solution of the gum in M NaCl using a Beckman Model E Ultracentrifuge at 20,410 r.p.m., gave a symmetrical boundary as detected by Schlieren optics. The gum was chromatographed[16] on a "Sepharose-4B" column (40 × 2·5 cm); elution with M NaCl gave a single symmetrical peak. The gum was eluted (M NaCl), as a single peak, at the void volume of a "Bio Gel P-300" column (20 × 2·5 cm). The gum was also chromatographed[14] on a DEAE-cellulose column (30 × 1·5 cm); gradient elution with NaCl solution (0·00–0·50 M) yielded a single, slightly asymmetrical peak.

Separation and Characterization of Constituent Sugars

The gum (3 g) was hydrolysed with 2 N H_2SO_4 (200 ml) for 12 hr at 100°; the cooled solution was neutralized ($BaCO_3$), filtered, treated with Amberlite resin IR-120 (H^+), and concentrated to a syrup. The syrup was fractionated on a Duolite A-4 resin column (30 × 1·5 cm) in the formate form. The neutral sugars were eluted with water (2 l.) and concentrated to a syrup (2·5 g). The acidic fraction was eluted with 5% formic acid (500 ml) and concentrated to a syrup (200 mg).

A portion of the neutral syrup was fractionated on Whatman No. 3MM sheets for 40 hr using solvent (*c*) to yield four fractions: (i) D-galactose (220 mg) had m.p. and mixed m.p. 167–168°, $[\alpha]_D$ +80° (*c.* 1·05, water); (ii) D-mannose (35 mg) had m.p. and mixed m.p. 131–132°, $[\alpha]_D$ +15° (*c.* 0·70, water); (iii) L-arabinose (510 mg had m.p. and mixed m.p. 159°, $[\alpha]_D$ +104° (*c.* 1·02, water); and (iv) D-xylose (41 mg) had m.p. and mixed m.p. 144–146°, $[\alpha]_D$ +20° (*c.* 0·82, water). A portion of the neutral syrup was fractionated on Whatman No. 3MM sheets for 16 hr using solvent (*a*). Elution of the appropriate zone with water gave L-rhamnose (20 mg) having m.p. and mixed m.p. 94°, $[\alpha]_D$ +8° (*c.* 0·40, water).

Chromatography of the acidic syrup using solvents (*c*) and (*d*) indicated the presence of D-glucurono-6,3-lactone, D-glucuronic acid, and traces of unhydrolysed aldobiouronic acids. D-Glucurono-6,3-lactone (36 mg) was isolated by preparative paper chromatography using solvent (*c*), and had m.p. and mixed m.p. 177°, and $[\alpha]_D$ +19° (*c.* 0·36, water).

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[22] D. M. W. ANDERSON and S. RAHMAN, *Carbohyd. Res.* **4**, 298 (1967).
[23] D. M. W. ANDERSON, SIR EDMUND HIRST, S. RAHMAN and G. STAINSBY, *Carbohyd. Res.* **3**, 308 (1967).
[24] A. B. S. CONACHER and D. I. REES, *Analyst* **91**, 55 (1966).