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Note

Identification of melezitose and erlose in floral and honeydew honeys

FLAVIO BELLIARDO, MARIA BUFFA,
Istituto di Chimica Farmaceutica e Tossicologica, Università di Torino, Turin (Italy)
AUGUSTO PATETTA, AND AULO MANINO
Istituto di Entomologia agraria e Apicoltura, Università di Torino, Turin (Italy)

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Although many techniques have been described for the separation and identification of oligosaccharides, the analysis of sugar components having d p (degree of polymerization) >2 in complex biological materials still cannot be achieved easily by means of the chromatographic techniques now available Other methods, such as total and partial hydrolysis, must be used to supplement chromatographic data

The purpose of this study was to investigate the presence of melezitose and/or erlose in floral and honeydew honeys The methodology developed will also be useful to apiculturists in evaluating honey samples collected from evergreens and from some deciduous trees

Both thin-layer and gas–liquid chromatography were used for preliminary identification, the structures of the main components of the two trisaccharide fractions were studied by enzymic and acid hydrolysis on the t l c layer of silica gel In this way, the main trisaccharide in the flower honey was shown to be erlose, and that in the honeydew honey to be melezitose

Tabulated data for t l c separations of the two honey samples under investigation are reported in Table I Fructose, glucose, sucrose, turanose, maltose, and a trisaccharide fraction were detected in both honey types Fig 1 shows gas chromatograms for the two samples the main peak of the two trisaccharide fractions has the same retention time as that of melezitose, a small peak, corresponding to what appears to be raffinose, can also be seen

During t l c on silica gel, the trisaccharide fraction migrated as a single band with an hR_F-value* in the range 9 2–9 8 for *Robinia* honey and 8 6–8 9 for honeydew honey. The behaviour with diphenylamine aniline phosphate, thiobarbituric acid,

*hR_F = 100 × R_F True R_F values after a single development were calculated from apparent data obtained after three developments, according to the procedure of French and Wild[1]

TABLE I

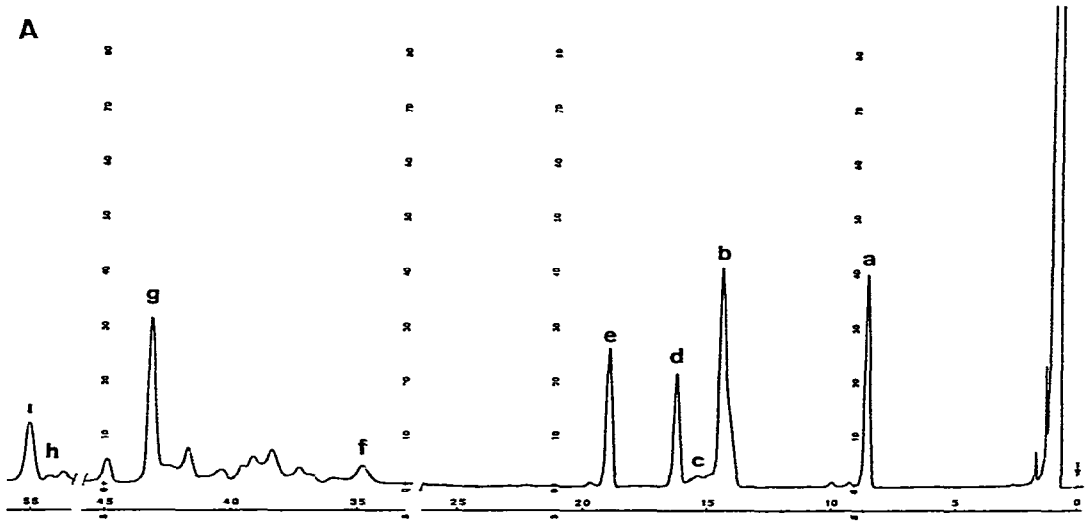
T L C DATA[a] FOR THE SUGAR COMPONENTS OF FLORAL AND HONEYDEW HONEYS

Sugars	*hR$_F$ Values*[b]		
	Standards	*Floral honey*	*Honeydew honey*
L-Rhamnose	33–34 (8)	—	—
D-Ribose	32–33 (8)	—	—
D-Xylose	29–30 (8)	—	—
D-Fructose	24–25 (8)	24–25 (9)	24–24 5 (9)
D-Glucose	21–23 (8)	22–23 (9)	22–23 (9)
D-Galactose	20–21 (8)	20 5–21 (9)	20 5–21 (9)
Sucrose	15–17 (8)	15–17 (9)	15–17 (9)
Turanose	13–14 (8)	—	—
Maltose	12–13 (8)	12 5–13 (9)	12 5–13 (9)
Melibiose	10–11 (8)	—	—
Erlose	9 2–9 5 (8)	9 2–9 8 (9)	—
Melezitose	8 5–8 9 (8)	—	8 6–8 9 (9)
Raffinose	7 6–8 (8)	—	—
Stachyose	3 4–3 9 (8)	—	—

[a]The values in parenthesis indicate the number of experimental values used to determine the hR_F values [b]hR_F = 100 × R_F

and 2-biphenylamine spray reagents demonstrated that the trisaccharide components in both samples contained ketose together with aldohexose residues

β-D-Fructosidase split D-fructose from the trisaccharide of *Robinia* honey, giving maltose, but was inactive on the main trisaccharide component from honeydew honey This showed that the D-fructose residue was terminal in the former case, and not terminal in the latter



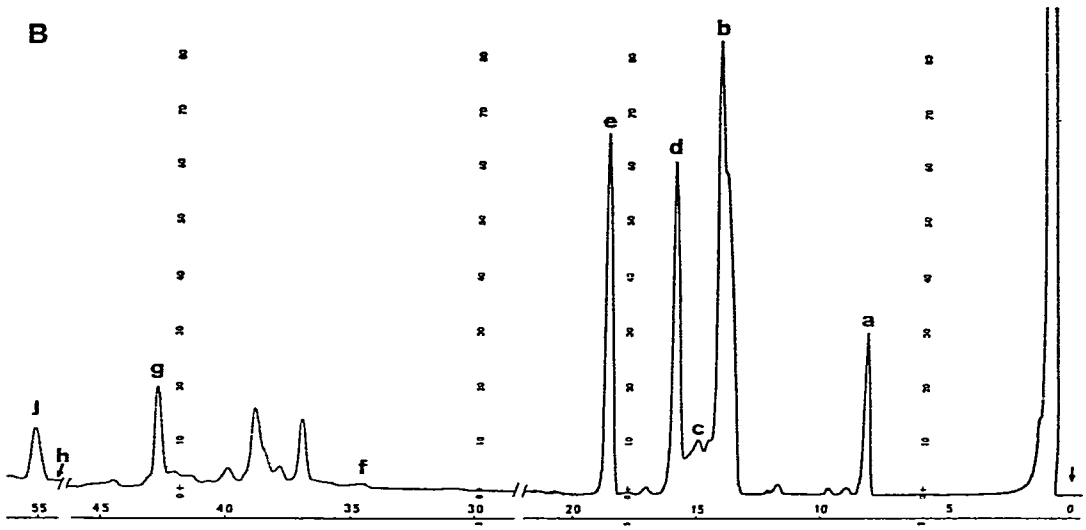


Fig 1 Gas chromatograms of the soluble carbohydrates from honeydew honey (A) and *Robinia pseudoacacia* L honey (B) a, arabinose (internal standard), b, α-fructose, c, β-fructose, d, α-glucose, e, β-glucose, f, sucrose, g, gentiobiose (internal standard), n, raffinose, i, melezitose, and j, erlose

On the basis of t l c , g l c , and hydrolysis results, it is therefore concluded that the main trisaccharide component of honeydew honey is melezitose, whereas the main trisaccharide component in the *Robinia* honey is erlose [*O*-α-D-Glc*p*-(1→4)-*O*-α-D-Glc*p*-(1→2)-β-D-Fru*f*]

EXPERIMENTAL

The samples of honey used in these studies were removed from a honey-bee colony foraging on *Robinia pseudoacacia* L and from a colony gathering mainly honeydew from linden leaves (*Tilia cordata* Miller) Before chromatographic analysis, all samples were subjected to melissopalynologic examination to determine the true botanic origin

Thin-layer chromatography — T l c was performed on standard (20 × 20 cm) plates precoated with Kieselgel G (Merck, layer thickness, 250 μm), 10–15 μl of a 30 mg/ml solution of the freeze-dried material were spotted on the plate The solvent system was chloroform–acetic acid–water[2] (6 7 1), 3 ascents at 28° Reference sugars were applied as 2 μl of solution (5 mg/ml) in 70% ethanol The spots were rendered visible with various spray reagents diphenylamine aniline phosphate, thiobarbituric acid, triphenyltetrazolium chloride, and 2-biphenylamine[3]

Acid and enzymic hydrolysis — These hydrolyses were carried out by the *in situ* technique[4]. Acid hydrolysis was effected by spraying the layer with 0 25M hydrochloric acid and heating the plate at 80° for 30 min in an "S"-chamber Enzymic hydrolysis was carried out by spraying the layer with a 0 01% solution of β-D-fructosidase (Boehringer, Mannheim, G F R) and incubating the plate for 30 min at

37° under constant humidity. Enzyme action was stopped by heating the plate at 100° for 3 min

Gas–liquid chromatography. — G l c was carried out on a Fractovap 2400 V (Carlo Erba) dual-column chromatograph, fitted with hydrogen-flame detectors, temperature-programming unit, recorder, and an electronic digital integrator Standard, twin, glass U-columns (3 mm × 2 m) packed with 3% of OV-101 on GCP S (80–100 mesh) were used The separations obtained in this report were generally achieved under the following conditions initial nitrogen flow-rate, 25 ml/min, hydrogen flow-rate, 45 ml/min, air flow-rate, 400 ml/min, and linear temperature programming at 3°/min (150→300°) The trimethylsilyl derivatives were prepared by the method of Ellis[5]

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