mammalian microsomal EH by an epoxide-containing but not a structurally related pyrrolizidine alkaloid toxin of rangeland weeds[20], may have important implications for grazing livestock. Tiger moths that consume *Senecio* alkaloids preferentially sequester the unsaturated analogs at the expense of the epoxide and diol bearing alkaloids[8]. Possibly an EH is used by these arctiids to egest more autotoxic pyrrolizidine alkaloids and thus retain the remainder for aposematic defenses.

Terpenoid epoxides and their precursor olefins represent another group of phytochemicals widely consumed by arthropods[6]. Many have high biological activity and are particularly abundant in the Compositae. This may explain the high EH activity of the *Solidago* specialist, *Trirhabda virgata* (table). Indeed, insects require sesquiterpene epoxides called juvenile hormones for growth regulation which are readily deactivated by insect EHs presumably similar or identical to the trans-EH characterized in mammals[12,18,19].

Plants can counter insect herbivory by synthesizing juvenile hormone mimics or antagonists of the hormone's biosynthesis or degradation within insects. Some of these defensive chemicals are terpenoid epoxides[21]. Perhaps the ubiquitous flavonoids of plants also disrupt insect endocrinology, since potent inhibitors of the trans-EH in mammals are synthetic derivatives of flavonoids[19].

Inspection of the table suggests that EH may be useful in biochemotaxonomy of arthropods. EH profiles among species of chrysomelids in the same genus are more similar than species from other genera in the same tribe, and the relatedness decreases as expected from the tribe to subfamily levels. Predaceous coccinellids from the same tribe (Cn, Hg, Cm) had a significantly different enzyme profile from that of a herbivorous coccinellid in another tribe (Ev, fig.). More species need to be examined with additional epoxide substrates to clarify the utility of this enzyme for distinguishing species with similar morphological characters.

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2 Miller, J.R., and Strickler, K.L., in: Chemical ecology of insects. Eds W.J. Bell and R.T. Cardé. Chapman and Hall, London 1984, in press.
3 Hsiao, T.H., and Fraenkel, G., Ann. ent. Soc. Am. *61* (1968) 493.
4 Chapman, R.F., Bull. ent. Res. *64* (1974) 339.
5 Brattsten, L.B., in: Herbivores, their interaction with secondary plant metabolites, p. 199. Eds G.A. Rosenthal and D.H. Janzen. Academic Press, New York 1979.
6 Mabry, T.J., and Gill, J.E., in: Herbivores, their interaction with secondary plant metabolites, p. 501. Eds G.A. Rosenthal and D.H. Janzen. Academic Press, New York 1979.
7 Kolattukudy, P.E., in: The biochemistry of plants, vol. 4, p. 571. Ed. P.K. Stumpf. Academic Press, New York 1980.
8 Rothschild, M., Aplin, R.T., Cockrum, P.A., Edgar, J.A., Fairweather, P., and Lees, R., Biol. J. Linnean Soc., Lond. *12* (1979) 305.
9 Nakanishi, K., Goto, T., Ito, S., Natori, S., and Nozoe, S., eds, Natural products chemistry. 2 vols. Academic Press, New York 1974-75.
10 Brooks, G.T., Gen. Pharmac. *8* (1977) 221.
11 Guenthner, T.M., and Oesch, F., in: Polycyclic hydrocarbons and cancer, vol. 3, p. 183. Eds H.V. Gelboin and P.O.P. Ts'o. Academic Press, New York 1981.
12 Hammock, B.D., and Quistad, G.B., Prog. Pestic. Biochem. *1* (1981) 1.
13 Mullin, C.A., Croft, B.A., Strickler, K., Matsumura, F., and Miller, J.R., Science *217* (1982) 1270.
14 Krieger, R.I., Feeny, P.P., and Wilkinson, C.F., Science *172* (1971) 579.
15 Croft, B.A., Phil. Trans. R. Soc. *B295* (1981) 125.
16 Mullin, C.A., Ph. D. thesis. Cornell University, Ithaca 1979.
17 Holloway, P.J., Brown, G.A., and Wattendorff, J., J. exp. Bot. *32* (1981) 1051.
18 Gill, S.S., and Hammock, B.D., Biochem. Pharmac. *30* (1981) 2111.
19 Mullin, C.A., and Hammock, B.D., Archs Biochem. Biophys. *216* (1982) 423.
20 Miranda, C.L., Cheeke, P.R., and Buhler, D.R., Res. Commun. chem. Path. Pharmac. *29* (1980) 573.
21 Bowers, W.S., Ent. exp. appl. *31* (1982) 3.
22 Wilcox, J.A., Leaf beetle host plants in northeastern North America. BRIA/World Nat. Hist. Publ. New York 1979.
23 Howe, W.L., and George, B.W., in: Insect colonization and mass production, p. 367. Ed. C.N. Smith. Academic Press, New York 1966.
24 Cinereski, J.E., and Chiang, H.C., J. econ. Ent. *61* (1968) 1531.
25 Gould, G.E., Bull. Indiana agric. Exp. Stn *490* (1944) 1.
26 Krysan, J.L., and Branson, T.F., Proc. 1982 int. Maize Virus Disease Workshop, Wooster, Ohio, in press.

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## Identification of raffinose in honeydew[1]

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*Summary.* The presence of small amounts of raffinose in the honeydew of 6 aphid species was demonstrated by means of TLC and GLC after invertase hydrolysis. The method allows the detection of this sugar even in the presence of a high percentage of melezitose.

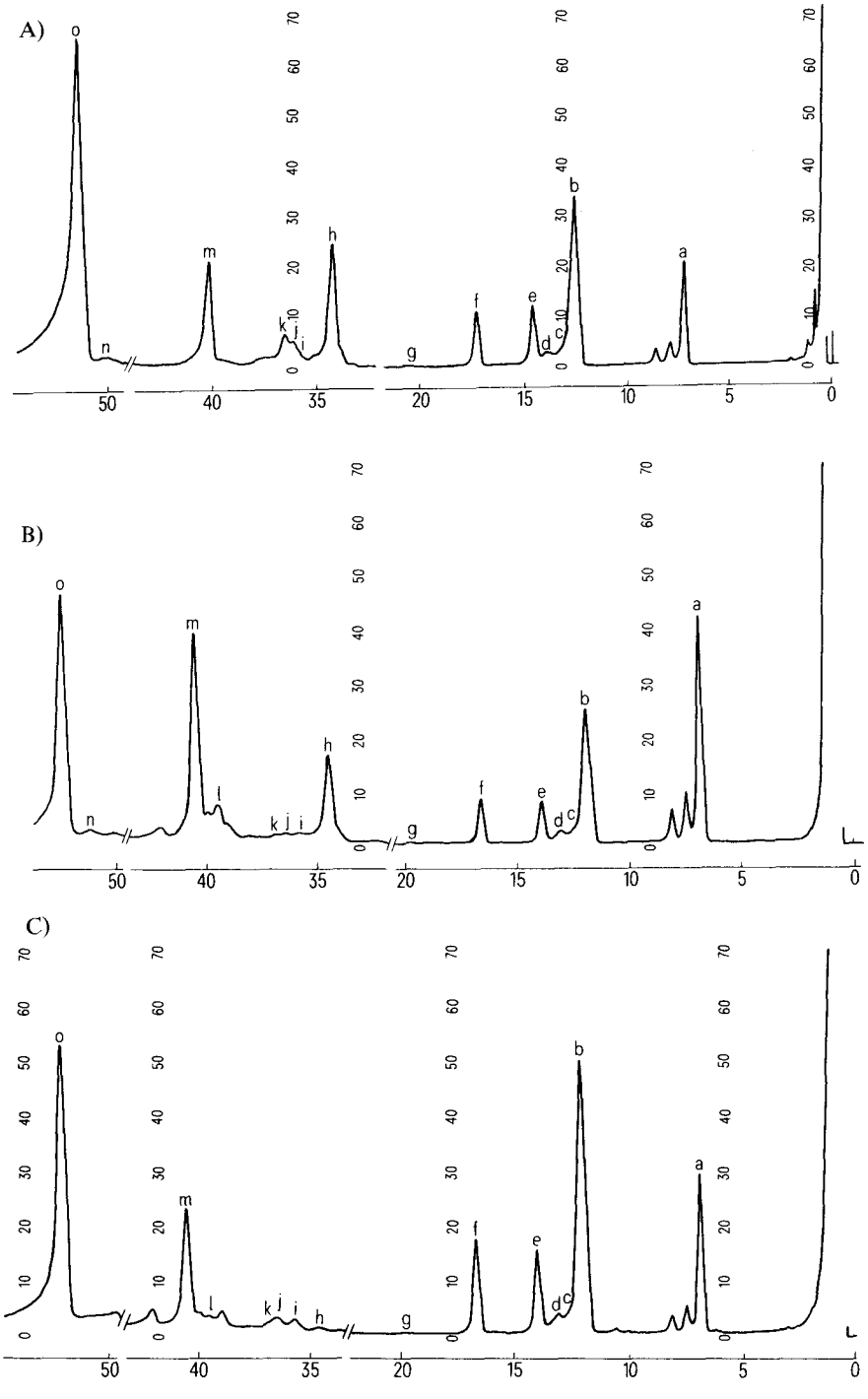
The honeydew produced by many aphids and the corresponding honeydew-honey are complex mixtures of mono-, di-, and oligosaccharides, often characterized by a very high melezitose content. The presence of raffinose in the trisaccharide fraction is usually accepted[2,3], but recently doubts have been raised as to whether it has been adequately demonstrated[4].

The aim of our work was to demonstrate the presence of

raffinose in the honeydews produced by some aphid species of great interest for honey production in Northern Italy, by TLC, GLC, and invertase hydrolysis.

TLC was used for preliminary analysis. Fructose, glucose, sucrose, disaccharides, trisaccharides, and in some cases higher molecular weight components were detected. Results obtained by GLC were consistent with TLC data; in addition, this method demonstrated the presence of galactose, mesoinositol, raffinose, and melezitose on the basis of retention time values and by the addition of internal standards. The GLC chromatograms obtained for *Cinara laricis* (Hartig, 1839) honeydew are reported in the figure.

The presence of raffinose was confirmed by means of TLC and GLC analysis of invertase hydrolysates of the honeydews. The hydrolysis was performed in the test tube and not by the in situ technique[5], in order to obtain a larger amount of hydrolysate. Raffinose was hydrolyzed by the enzyme, giving melibiose and fructose, while melezitose was not. In the GLC chromatograms, the appearance, or the increase, of the melibiose peaks was accompanied by the disappearance of the raffinose peak. Both chromatographic methods clearly resolved melibiose, the product of hydrolysis of raffinose, and isomaltose, the expected product if theanderose was present. There can be no doubt, therefore, as to the identity of the raffinose.




Gas chromatograms of the soluble carbohydrates of *Cinara laricis* (Htg.) honeydew (A), *C. laricis* honeydew with the addition of melibiose (B), and invertase hydrolysate of *C. laricis* honeydew (C); a, arabinose (internal standard); b, $\alpha$-fructose; c, $\beta$-fructose; d, galactose; e, $\alpha$-glucose; f, $\beta$-glucose; g, mesoinositol; h, sucrose; i, maltose; j, turanose; k, trehalose; l, melibiose; m, gentiobiose (internal standard); n, raffinose; o, melezitose.

The results obtained show that the use of the 2 techniques of enzymic hydrolysis and of chromatographic separation (TLC and GLC) can usefully aid in demonstrating the presence of a minor oligosaccharide component in a complex sugar mixture.

‰ *Experimental.* The analyses were carried out on samples the honeydews produced by the following aphids and collected in the years 1978–1981: *C. laricis* on *Larix decidua* Mill., *Tuberolachnus salignus* (Gmelin, 1799) on *Salix* sp., *Chaitophorus tremulae* Koch, 1854 on *Populus tremula* L., *Eucallipterus tiliae* (Linnaeus, 1758) on *Tilia cordata* Mill., *Myzocallis castanicola* Baker, 1917 on *Castanea sativa* Mill., *Aphis craccivora* Koch, 1854 on *Robinia pseudoacacia* L. Reference sugars were obtained from commercial sources.

TLC was performed on standard 20 × 20 cm plates, coated with Kieselgel G (Merck, Darmstadt, FGR), layer thickness 250 μm, with the solvent system chloroform-acetic acid-water (6:7:1), as described in a preceding paper[6]. 15 μl of a 2.5% aqueous solution of freeze-dried material was applied on the plate in 2 cm bands. The spots were detected with the spray reagent diphenylamine aniline phosphate.

GLC was carried out on a Fractovap 2400 V (Carlo Erba, Milano, Italy) dual-column chromatograph, fitted with hydrogen-flame detectors, temperature programming unit, recorder, and electronic digital integrator. Standard twin glass U-columns (3 mm × 2 m) packed with 3% OV 101 on GCP S (100–120 mesh) were used. The separations were generally achieved under the following conditions: initial nitrogen flow-rate, 25 ml/min; air flow-rate, 400 ml/min; linear temperature programming at 3 °C/min (150–300 °C). The trimethylsilyl derivatives were prepared by Ellis's method[7].

Hydrolysis with β-D-fructosidase (Boehringer, Mannheim, FGR) was carried out in the test tube with a 0.01% aqueous solution of the enzyme (30 min at 37 °C), stopping enzyme action by heating for 3 min at 100 °C. Hydrolysates were analyzed by TLC and GLC under the conditions described above.

TLC data for the sugar components of the investigated honeydews and of their invertase hydrolysates. The values in parentheses indicate the number of experimental data used in the determination of the $hR_{Glc}$ values ($hR_{Glc} = 100 \times R_F$ relative to glucose) for each sample

| | Standards | Honeydew | Invertase hydrolysate |
|---|---|---|---|
| D-fructose | 103–112 (10) | 107–113 (12) | 110–114 (6) |
| D-glucose | 100 | 100 | 100 |
| D-galactose | 92–94 (10) | a | a |
| Sucrose | 73–77 (10) | 72-76 (12) | – |
| Maltose | 58–62 (10) | | |
| Turanose | 56–60 (10) | 57–65[b] (12) | 56–61[b] (6) |
| Isomaltose | 55–57 (10) | – | – |
| Trehalose | 54–58 (10) | 54–58 (5) | c |
| Melibiose | 44–48 (10) | – | 44–49 (5) |
| Raffinose | 30–37 (10) | 34–37[b] (12) | 34–38[b] (6) |
| Melezitose | 32–35 (10) | | |
| Tetrasaccharides[d] | 12–15 (10) | 15–20 (12) | 14–18 (5) |

[a] When present in small quantities, galactose is partially masked by glucose. [b] Due to the very close migration rates, the 2 components are not resolved. [c] The corresponding band, too weak, was not sufficiently defined. [d] The standard was stachyose.

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2 Maurizio, A., in: Honey, p.95. Ed. E. Crane. Heinemann, London 1975.
3 Hadorn, H., Zürcher, K., and Strack, C., Mitt. Geb. Lebensmittelunters. Hyg. *65* (1974) 198.
4 Siddiqui, I.R., Adv. Carbohyd. Chem. Biochem. *25* (1970) 285.
5 Lombard, A., Tourn, M.L., and Buffa, M., J. Chromat. *134* (1977) 242.
6 Belliardo, F., Buffa, M., Patetta, A., and Manino, A., Carbohyd. Res. *71* (1979) 335.
7 Ellis, W.C., J. Chromat. *41* (1969) 325.

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## Dinemorphan N-demethylation by mouse liver microsomes

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*Summary.* Dinemorphan, an antitussive drug, is N-demethylated in vitro by mouse liver microsomes with biphasic kinetics showing two apparent $K_m$ and $V_{max}$. Moreover, dinemorphan N-demethylation is inhibited by CO, SKF-525A, metyrapone and it is specifically catalyzed by a phenobarbital-inducible form of cytochrome P-450.

Dinemorphan (d-3-methyl-N-methylmorphinan) phosphate (fig. 1) is an antitussive drug, supplied commercially as Astomin®, which has a structure and properties similar to the well known drug, dextromethorphan[2].

In the literature there are no reports on the metabolism of dinemorphan, either in vivo or in vitro. However, when dinemorphan was administered in vivo to several species, N-demethylated metabolites were found in the urine (Yamanouchi Pharm. Co., private communication). The N-demethylation of dextromethorphan has been studied but only in postmitochondrial fractions[3,4]. The present paper is concerned with the N-demethylation of dinemorphan and its kinetic parameters, using liver microsomes from mice treated with various inducers.

*Materials and methods.* 7–9-week-old male mice (25–35 g) of the Swiss albino CD1 strain (Gentili, Pisa) were housed in clear plastic cages with a bedding of wood shaving and were provided with tap water and food pellets (Altromin®; Rieper, Bolzano, Italy) ad libitum. The mice, when induced, were treated with phenobarbital (PB; 80 mg/kg, i.p.) in saline for 3 days prior to being killed (24 h after final injection), or with a single i.p. injection of 3-methylcholanthrene (3-MC; 80 mg/kg) in corn oil 48 h before sacrifice. Hepatic microsomes were prepared as previously