3,4-Di-*O*-isobutyryl-6-*O*-caprylsucrose: the Major Component of a Novel Sucrose Ester Complex from the Type B Glandular Trichomes of *Solanum berthaultii* Hawkes (PI 473340)

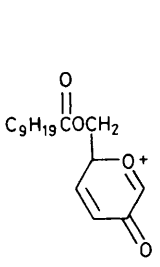
Russell R. King,*[a] Yvan Pelletier,[a] Rudra P. Singh,[a] and Larry A. Calhoun[b]

[a] *Research Station, Research Branch, Agriculture Canada, Fredericton, New Brunswick, Canada E3B 4Z7*
[b] *Department of Chemistry, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6E1*

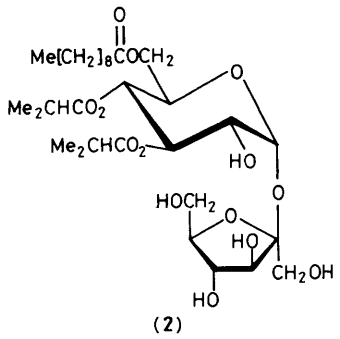
Evidence is presented that sucrose esters comprise the major portion of nonvolatile constituents in the exudate from type B glandular trichomes of *Solanum berthaultii* Hawkes (PI 473340); the principal sucrose ester was characterized as 3,4-di-*O*-isobutyryl-6-*O*-caprylsucrose.

The foliage of many wild potato species is covered with glandular trichomes (type A and type B) that utilize chemical secretions to entrap arthropod pests.[1,2] Type A trichomes release a quick setting fluid when ruptured but the type B constantly exude droplets of a clear sticky substance. Previous investigations[2] involving the exudate from type B trichomes of *Solanum berthaultii* Hawkes confirmed the presence of numerous volatile sesquiterpenes including the aphid alarm pheromone (*E*)-β-farnesene. We have now identified a complex of sucrose esters that comprise the major portion of nonvolatile constituents in the exudate from type B glandular trichomes of *S. berthaultii* Hawkes (PI 473340). Although fatty acid esters of sucrose are widely utilized in the food, detergent, pharmaceutical, and polymer industries,[3] to our knowledge only one other naturally occurring sucrose ester complex has been reported[4] and that a tetracylated mixture extracted from green tobacco leaves.

The isolation procedure involved initial chloroform extraction of the leaves (5 ml/g), and sequential fractionation of the nonvolatile residues by preparative t.l.c. For clone 1726 of *S. berthaultii* Hawkes (PI 473340)[5] (which contains an abundance of type B hairs) this procedure (0.5 mm silica gel 60 with chloroform–methanol 6:1) yielded a clear viscous fraction (R_f 0.47) that constituted the bulk of recovered



(1)



(2)

materials. (In crosses derived from clone 1726, progeny lacking type B hairs did not yield this viscous material.)

^{13}C N.m.r.[6,7] and two-dimensional ^{1}H n.m.r.[8] spectra of the isolate indicated the presence of sucrose molecules esterified at the C-3, C-4, and C-6 positions of the glucose moieties. These assignments were substantiated by subjecting samples of the isolate to; (i) enzymatic hydrolysis with invertase and (ii) transesterification with sodium methoxide. The enzymatic hydrolysis yielded fructose and a complex of periodate susceptible trisubstituted glucose esters. Transesterification with sodium methoxide afforded sucrose and a mixture of C_4, C_5, and C_{10} methylated fatty acids (capric and isobutyric were the major acids, isocapric and 2-methylbutyric the minor). Molecular ions for the sucrose ester complex were not obtained by direct probe mass spectra [electron impact (E.I.) mode]. Predictably[9] only high mass ions (*i.e.* *m/z* 457 and 471) corresponding to those observed for the invertase derived glucose moieties were obtained. Subsequent capillary g.l.c.–mass spectra (m.s.) (E.I.) of the sucrose esters as their acetyl derivatives indicated the presence of one major and five significant minor components (acetyl derivatives proved superior to the trimethylsilyl ethers because they were better resolved on g.l.c. and C_3H_7Si ions would have interfered with the detection of butyryl substituents). The parent components were assigned letters based on the order of elution of their acetyl derivatives by g.l.c. on a DB-5 capillary column. High mass ions of the acetyl derivatives and their relative proportion of the complex were as follows: for (A), *m/z* 499 (18.4%); (B), *m/z* 499 (43.7%); (C), *m/z* 513 (4.1%); (D), *m/z* 513 (8.3%); (E) *m/z* 513 (14.9%); (F) *m/z* 513 (7.5%). Mass ions for the major acyl groups, *i.e.*, butyryl *m/z* 71 and capryl *m/z* 155, were prominent in all six components. A mass ion for methylbutyryl groups (*m/z* 85) was prominent only in components (C), (D), (E), and (F).

In their respective mass profiles three of the four common prominent higher mass ions (*i.e.* *m/z* 169, 211, and 331) could be ascribed to degradated elements of an acetylated fructofuranosyl moiety.[10] The remnant ion (*m/z* 281) if representative of the pyroxonium structure (**1**) establishes[9,7] the presence of a capryl moiety at the C-6 position in all six components. Support for this assignment was demonstrated when partial hydrolysis of the complex with methanolic ammonia afforded a near quantitative mixture of disubstituted (at C-4 and C-6) and monosusbstituted (at C-6) sucrose esters in which the capryl groups were still present. Prolonged hydrolysis increased the yield of the monosubstituted derivatives at the expense of the disubstituted ones.

On the basis of the evidence presented, compounds (A) and (B) must contain isobutyryl substituents at C-3 and C-4. The compounds would differ only in the structure of their capryl moieties at C-6. Likewise, compounds (C), (D), (E), and (F) must consist of C-6 isomeric caprates differing in an interchange of isobutyryl and 2-methylbutyryl groups at the C-3 and C-4 positions.

Structural designations for the complex were further elucidated by the separation and characterization of compound (B) (the major component) as 3,4-di-*O*-isobutyryl-6-*O*-caprylsucrose (**2**). The separation was achieved by successive fractionations of the complex on 0.2 mm RP-C_{18} t.l.c. plates (acetone–H_2O 7:3). A fast atom bombardment (f.a.b.) positive ion spectrum[11] of the homogeneous isolate (R_f 0.45) exhibited a high mass peak at *m/z* 659.4 ($M + Na^+$) corresponding to the molecular formula $C_{30}H_{52}O_{14}$. Other significant peaks in the mass spectrum were observed at *m/z* 457 (14.7%), 369 (8.0%), 281 (21.3%) 155 (100%), and 127 (70.6%). The 200 MHz two-dimensional ^{1}H n.m.r. spectrum ($CDCl_3$) showed discrete signals for 1-H, 3-H, 4-H and 6-H at δ 5.51 (d, $J_{1,2}$ 4.1 Hz) 5.32 (t, $J_{3,4}$ 9.7 Hz) 5.11 (t, $J_{4,5}$ 9.7 Hz) and 4.15 (m) respectively. The 50 MHz ^{13}C n.m.r. spectrum ($CDCl_3$) allowed the assignment of signals at δ 60.59 (t), 60.99 (t), 64.28 (t), 66.89 (d), 68.83 (d), 70.57 (d), 73.27 (d), 73.50 (d), 80.00 (d), 82.05 (d), 92.32 (d), and 103.99 (s) corresponding to the sucrose moiety, signals at δ 14.13 (q), 22.65 (t), 31.88 (t), 29.15 (t), 29.29 (2t), 29.45 (t), 29.54 (t), 24.68 (t), 33.93 (t), and 173.68 (s) corresponding to C-10 through C-1 of the capryl group and signals at δ 18.84 (4q) for C-3 and C-3′, 33.85 (d), and 34.18 (d) for C-2, 175.35 (s) and 178.32 (s) for C-1 of the isobutyryl groups. G.l.c.–m.s. of the transesterified acid substituents confirmed the presence of methyl isobutyrate and methyl caprate in a distinct 2 to 1 ratio. Acetylation of compound (**2**) with acetic anhydride–pyridine and crystallization of the product from aqueous ethanol furnished the pure penta-acetate as colourless needles with m.p. 63—64 °C. The molecular formula was confirmed as $C_{40}H_{62}O_{19}$ on the basis of f.a.b. high resolution m.s., which gave *m/z* 846.443, calculated for 846.532. ^{13}C and ^{1}H n.m.r. spectra of the penta-acetate were consistent with the assigned structure.

Bioactivity studies[12] involving the sucrose esters indicate that their presence may contribute to the high levels of resistance to infection from *Phytophthora infestans* exhibited by clone 1726 of *S. berthaultii* (PI 473340).

We thank Jacques Langevin, C.B.R.I. Ottawa, and S. H. Tan, R. P. C., Fredericton, for the mass spectral determinations.

Received, 3rd March 1986; Com. 287

References

1 R. W. Gibson, *Potato Res.*, 1976, **19**, 179.
2 R. W. Gibson and J. A. Pickett, *Nature*, 1983, **302**, 608.
3 R. Khan, *Pure Appl. Chem.*, 1984, **56**, 833; R. J. Jandacek, *Int. J. Obesity*, 1984, **8**, Suppl. 1, 13; N. B. Desai and N. Lowicki, *Cosmet. Toiletries*, 1985, **100**, 55.
4 R. F. Severson, R. F. Arrendale, O. T. Chortyk, C. R. Green, F. A. Thomas, J. L. Stewart, and A. W. Johnson, *J. Agric. Food Chem.*, 1985, **33**, 870.
5 R. P. Singh, *Phytopathology*, 1985, **75**, 1432.
6 A. Allerhand and D. Doddrell, *J. Am. Chem. Soc.*, 1971, **97**, 2777.
7 D. M. Clode, W. A. Laurie, D. McHale, and J. B. Sheridan, *Carbohydr. Res.*, 1985, **139**, 161.
8 A. Bax, R. Freeman, and G. Morris, *J. Magn. Reson.*, 1981, **42**, 164.
9 R. Khan, *Adv. Carbohydr. Chem. Biochem.*, 1976, **33**, 278.
10 M. G. Lindley, G. G. Birch, and R. Khan, *Carbohydr. Res.*, 1975, **43**, 360.
11 A. Dell and C. E. Balou, *Carbohydr. Res.*, 1983, **120**, 95.
12 J. D. Holley, R. P.Singh, and R. R. King, *Can. J. Plant Pathol.*, 1986, in the press.