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Preliminary Studies of the Moody and Galva Soil Series of Northwestern Iowa

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The Galactan Series of Oligosaccharides[1]

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In most biochemistry text books, stachyose is given as the classic example for a tetrasaccharide. Some books list for a pentasaccharide, verbascose. Stachyose was first described in 1890 by von Planta and Schulze (1), who first isolated it and crystallized it from the rhizomes of *Stachys tuberifera*, the Japanese artichoke. They described it as a non-reducing trisaccharide, $[\alpha]_D = +148°$, composed of fructose, glucose and galactose. In 1903, Tanret (2) established stachyose as a tetrasaccharide when he showed that it was identical with what he had named manneotetrose from ash manna (3).

Since that time, stachyose has been isolated from many different plants. Those of the family, *Labiatae*, include: *Ajugoides humilis* (4), *Ballota faetida* (5), *Clinopodium chinense* var. *parviflorum* (4), *C. confine* (4), *C. vulgare* (5), *Dracocephalum argunense* (4), *Eremostachys laciniata* (7), *Lamium album* (6), *Leonurus sibiricus* (4), *Lycopus Maackianus* (4), *Mentha haplocalyx* (4), *M. sylvestris* (5), *Nepeta Glechoma* (4), *Origanum vulgare* (5), *Prunella asiatica* (4), *Salvia chinensis* (4), *S. nipponica* (4), *S. pratensis* (5), *S. splendens* (5), *Stachys lanata* (5), *S. riederi* var. *hispidula* (4), *S. recta* (5), *S. sylvatica* (5) and *S. tuberifera* (1).

The members of the family, *Leguminosae*, from which stachyose has been isolated include: *Coronilla scorpioides* and *C. varia* (8), *Ervum lens* (9), a *Dolichos* species (31), *Galega officinalis* (9), *Gleditsia tricanthos* (31), *Indigofera tinctoria* (31), *Leucaena glauca* (13), *Lupinus luteus* (14), *Medicago sativa* (31), *Phaseolus vulgaris* (15), *Pisum sativum* (16), *Soja hispida* (9), *Sophora japonica* (31), *Tetrapleura Thonningii* (17), *Trifolium incarnatum* (9) and *Trigonella feonum-graecum* (31).

It has been isolated from other species including: *Jasminum officinale* (18), *Fraxinus ornus* and *F. rotundifolia* (3) of the family, *Oleaceae; Scrophularia nodosa* and *S. sambucifolia* (19); *Catalpa bignonioides* (20); *Plantago maritima* and *P. carinata* (21); *Verbena officinalis* and *V. venosa* (22). It has been detected also in *V. bonariensis* and *V. hispida* (32).

Stachyose has also been reported to be in shoots from a young apple rootstock (SV411), from shoots of the Myrobalan B rootstock

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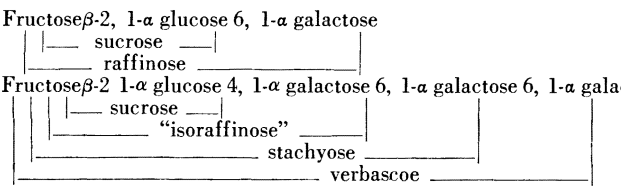
plum and from Conference pear prunings (23).

In our laboratory we have also detected stachyose in *Plantago major* L., *P. ovata*, *P. psyllium*, *P. rugelii* DC., *Stachys palustris* L., *S. tenuifolia* Willd., *Verbascum thapsus* L., *Teucrium canadense* L. and *Pueraria thunbergiana* Benth.

Verbascose $[\alpha]_D = +170°$, was discovered by Bourquelot and Bridel (12) in 1910. In our laboratory we have also detected verbascose in *Teucrium canadense* L., *Stachys palustris* L., *S. tenuifolia* Willd., *S. tuberifera* Naud., *Plantago major* L., *P. rugelii* DC., *Soja hispida* and in ash manna.

These two sugars, like raffinose, contain the sucrose grouping with α-galactosyl units attached to the glucose portion of the grouping. But they differ from raffinose, according to Onuki (10) and Murakami (11), in that the first galactose unit is bonded to carbon four of the glucose unit rather than carbon six as in raffinose. This seems rather strange since raffinose is the only known trisaccharide of this type from which one might expect these higher sugars to be derived. The structures of these sugars are reported as follows:



In view of this apparent irregularity, we decided to reinvestigate the structure of stachyose. Since no working amounts of stachyose were available, a source from which it could be isolated was sought. *Stachys palustris* L. was known to grow in central Iowa and was thought to be a likely source. Some rhizomes of what was thought to be *S. palustris* were gathered in the early spring of 1951 before growth began. Examination of an extract by paper chromatography showed that they contained a regular series of oligosaccharides up to and including high molecular weight material which did not move on the chromatogram. It was not until some time later that it was discovered that we had gathered *Teucrium canadense*. This appeared to be a promising source of stachyose. Subsequent examination of extracts from several *Stachys* species, *Plantago rugelii*, *P. major*, *Verbascum thapsus* and ash manna showed that the same series of oligosaccharides occurred in them, differing somewhat, however, in the relative abundance of the different sugars, and lacking the higher molecular weight material.

The multiple ascent technique of paper chromatography was used

(24) with solvent composed of 3 parts water, 4 parts pyridine, and 6 parts *n*-butanol (25). Spots of fructose containing sugars were brought out by spraying on a 0.1% solution of phloroglucinol in *n*-butanol which was 2M. in HCl, and heating in a 105°C. oven for about one minute. Reducing sugars were detected by spraying with alkaline copper reagent 60 without KIO_3 and KI (26), heating in a 105°C. oven for about five minutes, then spraying with phosphomolybdic acid reagent (27).

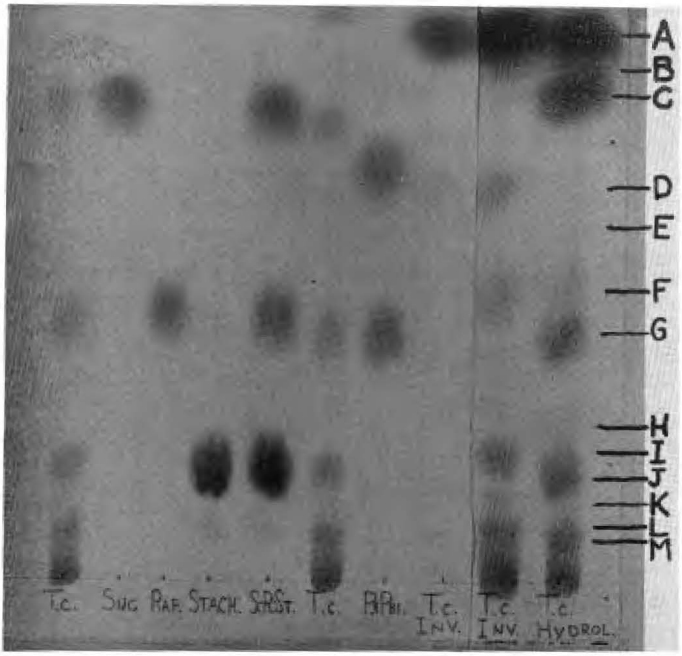


Figure 1. Paper Chromatogram of *Teucrium canadense* Carbohydrates. T.c.-*T. canadense* extract; Suc.-Sucrose; Raf.-Raffinose; Stach.-Stachyose; S.R.St.-Sucrose, raffinose and stachyose; P. & Pbi.-Planteose (lower) and planteobiose (upper); T.c. Inv.-Inverted T.c.; T.c. Hydrol.-More extensively hydrolyzed T.c.; A-Fructose, B-Glucose, C-Galactose, D-Planteobiose, E,H, & K-Uncertain, F-Melibiose, G-6Galactosyl-galactose, I-Manninotriose, J-Trigalactose cpd., L-Inv. verbascose tetrasacch., M-Tetragalactose cpd.

The apparent R_f values for the first few sugars in *T. canadense* after three ascents are sucrose 0.76, raffinose 0.42, stachyose 0.19, verbascose 0.08 with the higher sugars unresolved (see Fig. 1).

Two volumes of 95% ethanol were added to a 30% solution of the series and a white, granular precipitate of the high molecular weight material came down. A solution of the precipitated material was hydrolyzed with 0.3 M. H_2SO_4 at 100° C. for 20 minutes, and then neutralized (T.c. Hydrol.). A solution of the material which was not precipitated by the alcohol was inverted with 0.1 M. H_2SO_4

at 100° C. for 4 minutes and then neutralized (T.c. Inv.). Comparing the two hydrolysis mixtures, it is seen (Fig. 1) that fructose (R_f 0.89) stands out in each case. Galactose (R_f 0.79) is present in much larger amounts in the extensively hydrolyzed material; the small amount of glucose formed (R_f 0.84) is for the most part hidden by the fructose and galactose. Melibiose (R_f 0.48) is present in roughly equivalent amounts in the two mixtures. What is in all probability 6-(α-galactosyl) galactose (R_f 0.40) stands out in the more extensively hydrolyzed material, with but a trace in the inverted material. Manninotriose (R_f 0.21) stands out in the inverted material, but is unresolved from the trigalactose compound (R_f 0.17) in the hydrolyzed material. The reducing tetrasaccharide from the inversion of verbascose (R_f 0.09) is just resolved in the inverted material, but can be only approximated along with the tetragalactose compound (R_f 0.07) in the more extensively hydrolyzed series. The substance lettered "K" (R_f 0.14) in Fig. 1 may possibly be the inversion product from the sugar (maybe a pentasaccharide) which occurs to a minor extent between stachyose and verbascose on the chromatogram (R_f 0.13). The exact nature of this sugar is not yet known. The substances lettered "H" (R_f 0.27) and "E" (R_f 0.57) are as yet unidentified. They may contain linkages other than 1,6 linkages, which may be present in substances such as "K" or higher homologs.

Compound "D" (R_f 0.65) is planteobiose (28) which is a fructose containing disaccharide resulting from the inversion of planteose (R_f 0.40). Previous to this, planteose had been shown to be present only in plants of the genus, *Plantago* (29). Planteose is an isomer of raffinose, indistinguishable from it on the chromatogram since it has about the same R_f value and gives the same color with the phloroglucinol reagent.

Sucrose and raffinose have been separated from the series of carbohydrates from *T. canadense* by the method of Whistler and Durso (30). They have been crystalized and their identity established with X-ray powder patterns. Work is proceeding on the isolation of stachyose and the higher sugars.

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