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DISTRIBUTION OF SORBITOL IN ROSACEAE*

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Key Word Index—Rosaceae; sorbitol content; chemotaxonomy; cytotaxonomy; GLC of hexitols; silylation of hexitols.

Abstract—77 leaf samples representing 68 taxa of Rosaceae were investigated for the presence of sorbitol. A procedure for the quantitative estimation of sorbitol in dry plant tissues was elaborated; it made use of extraction by percolation and capillary GLC analysis of the silylated extracts. All Maloideae and Prunoideae and most Spiraeoideae were found to accumulate sorbitol. The subfamily Rosoideae was found to be heterogeneous in this respect; in most tribes sorbitol is totally lacking, but in Kerrieae, Adenostomeae and part of Dryadeae sorbitol is present in variable amounts. A clear-cut correlation between sorbitol accumulation and basic chromosome number seems to exist in Rosaceae.

INTRODUCTION

The sugar alcohol sorbitol was isolated for the first time from the fruits of *Sorbus aucuparia* (Rosaceae) by Boussingault [1] in 1872. Later sorbitol was detected in the fruits of many other members of this family [2]. At one time, the main stimulus to investigate plants, especially fruits, for the presence of sorbitol stemmed from its use in detecting the adulteration of fruit juices and other products derived from fruits [3]. The detection of sorbitol in a fruit product derived from fruits not containing sorbitol indicates its adulteration with cheap apples or pears. Later the dietetic significance of sorbitol stimulated research [4]. Sweet-tasting sorbitol is an important sugar substitute in the diet of diabetics.

Plouvier [5] was the first author to stress the importance of sorbitol for intra-familial classification of Rosaceae. The same author discussed the taxonomic significance of other sugar alcohols [6, 7], e.g., mannitol in Oleaceae and dulcitol in Celastraceae (inclusive of Hippocrateaceae). Plouvier [5] investigated 73 species of Rosaceae for the presence of sorbitol and observed that all Spiraeoideae, Maloideae and Prunoideae contained sorbitol and that most Rosoideae lacked detectable amounts of this hexitol. Species of *Rhodotypos*, *Kerria* and *Neviusia*, three genera included by Focke [8] and by Engler and Diels [9] in Rosoideae, however, were shown to contain sorbitol; this result supports those authors who exclude the tribe Kerrieae from the subfamily Rosoideae.

The aim of this investigation was to screen many rosaceous taxa for the presence of sorbitol and to evaluate the usefulness of this chemical character for taxonomic purposes within the family. Earlier investigators identified sorbitol mainly by extraction, purification by crystallization after acetylation or benzoylation and determination of melting points of the respective derivatives [3–5]. Later, paper chromatographic procedures were developed for this purpose [10–12]. In the present investigation capillary GLC was used to separate the TMS-derivatives of polyols from the monosaccharides [14–16] and to determine quantitatively sorbitol in plant extracts.

RESULTS AND DISCUSSION

The GLC procedure described in this paper allows quick and reliable qualitative and quantitative assays of sorbitol and isomeric hexitols. In conformity with Hansen and Møller [17] and Ericsson *et al.* [16], percolates prepared from small amounts of ground plant leaves can be used without further purification, because the silylated crude extracts do not contain compounds which interfere with the hexitol peaks under the conditions used. Ericsson *et al.* [16] did not delipidise the aqueous plant percolates. However, it is advisable to wash the concentrates with petroleum to remove interfering lipids.

There is little information in the literature about the sorbitol content of different plant parts. Strain [4] mainly investigated leaves and fruits of a number of rosaceous plants and recorded sorbitol yields, but Plouvier [5] reported in most instances only presence or absence. The present investigation considerably extends the knowledge of sorbitol accumulation in Rosaceae. Consequently this character can be used more reliably in future attempts to improve the intrafamilial classification of rosaceous taxa. The results are presented together with the results of Plouvier [5] and other investigators in Table 1. In order to relate a number of plant names to modern systems of nomenclature, several horticultural manuals were consulted [18–20]. Table 1 shows clearly, that sorbitol content represents a taxonomically useful character, within the family.

Twelve leaf samples of the Spiraeoideae were investigated, representing 10 species and 6 of the 7 tribes [21] of this subfamily. The results fully agree with those of Plouvier. The Quillajeae, not investigated by Plouvier contains little sorbitol in the leaves; only trace amounts were observed in *Quillaja brasiliensis* and small amounts in two samples of *Quillaja saponaria*. Quillajeae excepted, the sorbitol content varied between 1.3% (*Lyonothamnus*

* Part I in the series "Chemotaxonomy of Rosaceae".

Table 1. Distribution of sorbitol in Rosaceae

Taxa	Present paper* (% of dry leaves)	Results of Plouvier†	Recorded by others‡
Spiraeoideae			
Exochordeae			
Exochorda giraldii Hesse var. *wilsonii* Rehd.	11.3 (23762)	–	–
E. giraldii Hesse	–	+; l, br	–
Gillenieae			
Gillenia trifoliata (L.) Moench	6.0 (27100)	–	–
Holodisceae			
Holodiscus discolor (Pursh) Maxim.	–	+; l	–
Neillieae			
Neillia sinensis Oliv.	–	+; l	–
Physocarpus bracteatus (Rydb.) Rehd.	–	+; l	–
P. opulifolius (L.) Maxim.	6.6 (24617)	+; l, br, fr	–
Stephanandra tanakae Franch. et Sav.	–	+; l	–
Quillajeae			
Quillaja brasiliensis Mart.	0.08 (25743)	–	–
Q. saponaria Molina	0.2 (26288)	–	–
Q. saponaria Molina	0.5 (27098)	–	–
Sorbarieae			
Chamaebatiaria millefolium Maxim.	5.6 (76282)**	–	–
Lyonothamnus floribundus Gray var. *asplenifolius* (Greene) Bdg.	1.8 (26661)	–	–
L. floribundus Gray var. *floribundus*	1.3 (26662)	–	–
Sorbaria aitchisonii Hemsl.	11.6 (23763)	+; l, fr	–
S. sorbifolia (L.) A.Br.	–	+; l	–
Spiraeeae			
Aruncus dioecus (= *dioicus*) (Walter) Fernald (= *A. silvester* Kostel. = *Spiraea aruncus* L.)	3.0 (22134)	+; l	–
Sibiraea laevigata (L.) Maxim.	–	+; l	–
Spiraea bella Sims	3.9 (27058)	–	–
S. betulifolia Pall.	–	+; l	–
S. cantoniensis Lour.	–	+; l, br	–
S. chamaedryfolia L. –	–	+; l	
S. hypericifolia L.	–	+; l	–
S. japonica L.f.	–	+; l, fl	–
S. menziesii Hook.	–	+; l	–
S. prunifolia Sieb. et Zucc.	–	+; l	–
S. trilobata L.	–	+; l	–
Rosoideae			
Adenostomeae			
Adenostoma fasciculatum H. et A.	1.7 (26659)	–	–
A. sparsifolium Torr.	1.5 (26664)	–	–
Dryadeae			
–Cercocarpinae			
Cercocarpus betuloides Nutt.	<0.01 (26660)	–	–
C. ledifolius Nutt.	<0.01 (26665)	–	–
C. minutiflorus Abrams	<0.01 (26663)	–	–
—Dryadinae			
Dryas octopetala L.	<0.01 (16145)	–	–
– Geinae			
Coluria potentilloides (Dall.) Ledeb.	0.0 (26172)	–	–
Geum aleppicum Jacq.	0.0 (8785)	–	–
G. urbanum L.	–	0; l	–
Waldsteinia geoides Willd.	–	0; l	–
W. ternata (Stephan) Fritsch	0.0 (25355)	–	–
–Purshiinae			
Purshia tridentata DC.	0.4 (78262)**	–	–
Kerrieae			
Coleogyne ramosissima Torr.	9.8 (7082)	–	–

Table 1. (*continued*)

Taxa	Present paper* (% of dry leaves)	Results of Plouvier†	Recorded by others‡
Kerria japonica DC. var. *simplex* Boom	3.9 (23761)	–	–
K. japonica DC.	–	+; l, fl, br	–
Neviusia alabamensis Gray	–	+; l	–
Rhodotypos scandens (Thunb.) Makino	8.9 (27097)	+; l, br	–
Potentilleae			
–Alchemillinae			
Alchemilla alpina L. s. str.	0.0 (26548)	–	–
A. vulgaris L.	–	0; l	–
Aphanes microcarpa (Boiss. et Reut.) Rothm.	0.0 (18045)	–	–
–Potentillinae			
Bencomia caudata (Ait.) Webb et Berth	0.0 (24598)	–	–
Comarum palustre L. (= *Potentilla palustris* [L.] Scop.)	0.0 (18051)	0; l	–
Duchesnea indica (Andrews) Focke	0.0 (27052)	–	–
Fragaria × *ananassa* Duchesne (= *F. chiloensis* [L.] Duchesne × *F. virginiana* Duchesne)	0.0 (3652)	–	–
F. vesca L.	–	0; l	–
Potentilla anglica Laicharding	0.0 (18178)	–	–
P. fruticosa L.	–	0; l	–
Sibbaldia procumbens L.	0.0 (18296)	–	–
Roseae			
Rosa canina L.	0.0 (19983)	–	–
R. gymnocarpa Nutt.	–	–	0; fr [4]
R. hugonis Hemsl.	–	0; l	–
R. villosa L. (= *R. pomifera* Herrm.)	–	0; l	–
Rubeae			
Rubus caesius L.	0.0 (21476)	–	–
R. fruticosus L. s.l.	–	0; l	–
R. idaeus L.	–	0; l	–
Sanguisorbeae			
–Agrimoniinae			
Agrimonia asiatica Juz.	0.0 (26174)	–	–
A. eupatoria L.	–	0; l	–
Hagenia abyssinica J. F, Gmelin	0.0 (18164)	–	–
–Sanguisorbinae			
Acaena buchananii Hook. f.	–	0; l	–
Cliffortia crenata L. f.	0.0 (24744)	–	–
C. linearifolia E. et Z.	0.0 (24755)	–	–
Leucosidea sericea E. et A.	0.0 (24743)	–	–
Marcetella maderensis (Bornm.) Svent. (= *Sanguisorba maderensis* [Bornm.] Nordb.)	0.0 (24600)	–	–
Margyricarpus setosus Ruiz et Pav.	0.0 (24618)	–	–
Sanguisorba minor Scop. (= *Poterium sanguisorba* L.)	0.0 (22772)	0; l	–
S. officinalis L.		0; l	–
Sarcopoterium spinosum Spach. (= *Poterium spinosum* L.)	0.0 (21675)	–	–
Ulmarieae			
Filipendula ulmaria (L.) Maxim. (= *Ulmaria palustris* Moench = *Spiraea ulmaria* L.)	–	0; l	–
Filipendula vulgaris Moench (= *F. hexapetala* Gilib. = *Ulmaria filipendula* Hill = *Spiraea filipendula* L.)	0.0 (23936)	0; l	–
Maloideae			
Crataegeae			
Aronia melanocarpa (Michx) Ell.	2.4 (27051)	–	–
Cotoneaster affinis Lindl.	–	+; l, fr	–
C. bacillaris Lindl.	–	+; l, br, fr	–
C. dielsiana Pritz.	2.1 (18052)	–	–

Table 1. (*continued*)

Taxa	Present paper* (% of dry leaves)	Results of Plouvier†	Recorded by others‡
C. divaricata Rehd. et Wils.	–	+; l, fr	–
C. franchettii Bois	–	+; l, fr	–
C. frigida Wall.	–	–	2.7 fr [4]
C. horizontalis Decne.	–	–	2.1 fr [4]
C. integerrima Medic.	–	–	+; l [34]
C. microphylla Wall.	–	–	3.6 fr [4]
C. pannosa Franch.	–	–	5.1 fr [4]
C. salicifolia Franch.	–	+; l, fr	–
Crataegus azarolus L.	4.7 (18059)	–	–
Crataegus × *carrierei* Vauvel	–	+; l, fr	–
C. crus-galli L.	2.8 (18061)	+; l, fr	–
C. laevigata (Poir.) DC. (= *C. oxyacantha* L. emend. Jacq.)	2.4 (3092)	+; l, fr, br	7.6 fr [4]
C. laevigata (Poir.) DC.	4.7 (16275)	–	–
C. laevigata (Poir.) DC.	3.1 (24827)	–	–
C. monogyna Jacq.	3.6 (18062)	–	4.7 fr [4]
C. monogyna Jacq.	1.6 (18069)	–	–
C. monogyna Jacq.	5.0 (18070)	–	–
Mespilus germanica L.	4.6 (24514)	–	–
Pyracantha angustifolia (Franch.) Schneid.	–	–	4.7 fr [2, 3, 4, 12]
P. coccinea Roem.	–	+; l	–
P. crenulata (D. Don) Roem.	–	–	3.3 fr [2, 3, 4, 12]
× *Pyracomeles vilmorinii* Rehd.	–	+; l	–
Maleae			
Amelanchier canadensis (L.) Medic.	–	+; l, fr, br	–
A. florida Lindl.	–	+; l, fr	–
A. ovalis Medic.	2.2 (4628)	–	–
Chaenomeles lagenaria Koidz. (= *Ch. speciosa* [Sweet] Nakai)	–	+; l, fr	–
C. sinensis Koehne (= *Pseudocydonia sinensis* Schneid.)	–	+; l	–
Cydonia oblonga Mill. (= *C. vulgaris* Pers.)	0.7 (3667)	+; l, fr, br	–
C. oblonga Mill.	0.1 (3668)	–	–
Eriobotrya japonica (Thunb.) Lindl.	–	–	0.2 fr [4]
Heteromeles arbutifolia (Ait.) M. J. Roem. (= *Photinia arbutifolia* [Ait.] Lindl.)	–	–	0.9 l; 1.7 fr [4]
Malus pumila Poir. (= *M. communis* Poir. = *Pyrus malus* L. p. p.)	–	–	+; fr [2, 3, 4, 12]
M. silvestris (L.) Mill. (= *M. acerba* Mérat = *Pyrus malus* L. p. p.)	2.2 (12484)	–	0.45 l [4]
M. silvestris (L.) Mill.	3.1 (18161)	–	–
M. silvestris (L.) Mill.	2.0 (18162)	–	–
M. silvestris (L.) Mill.	3.6 (18163)	–	–
M. silvestris (L.) Mill.	3.2 (24055)	–	–
M. spectabilis (Ait.) Borkh.	–	+; l	–
Osteomeles anthyllidifolia Lindl.	–	+; l	–
Photinia serrulata Lindl.	–	+; l	–
Pyrus communis L.	8.7 (24078)	–	1.9–2.4 fr [2, 3]
P. salicifolia Pall.	–	+; l	–
Rhaphiolepis japonica Sieb. et Zucc. (= *Rh. umbellata* [Thunb.] Makino)	–	+; l, fr	–
Sorbus aria (L.) Crantz	–	+; l	–
S. aucuparia L.	–	+; l, br	10.0 fr [1, 2, 4]
S. chamaemespilus (L.) Crantz	8.4 (23139)	–	+; l [34]
S. commixta Hedl.	–	–	+; fr [2]
S. domestica L.	–	+; l, fr	–
S. sitchensis Roem.	–	–	6.1 fr [4]
S. torminalis (L.) Crantz	–	–	+; l [34]
Stranvaesia davidiana Decne. var. *salicifolia* (Hutchins.) Rehd.	5.9 (27055)	–	–

Table 1. (*continued*)

Taxa	Present paper* (% of dry leaves)	Results of Plouvier†	Recorded by others‡
Prunoideae			
Osmaronia cerasiformis (Torr. et Gray) Greene (= *Nuttallia cerasiformis* Torr. et Gray)	–	+; l, br, fr	–
Prinsepia sinensis Oliv.	16.8 (24641)	–	–
P. uniflora Batal.	–	+; l, fr	–
Prunus armeniaca L.	–	–	0.4 l [4]
P. avium L.	1.4 (19654A)	–	0.2 l [4]
P. cerasifera Ehrh.	–	+; l, br	–
P. cerasifera Ehrh. var. *pissardii* Koehne	–	+; l, br	–
P. fremontii Wats.	2.5 (19417)	–	–
P. ilicifolia (Nutt.) Walp.	0.4 (7071)	–	–
P. laurocerasus L.	0.3 (23757)	+; l	–
P. lusitanica L.	–	+; l	–
P. mahaleb L.	1.2 (10342)	+; l	–
P. padus L.	0.6 (6053)	–	–
P. persica (L.) Batsch	–	–	0.6 l [4]
P. serotina Ehrh.	0.7 (18212)	+; l	–
P. serrulata Lindl.	–	+; l	–
P. spinosa L.	3.6 (17825)	–	–
P. tomentosa Thunb.	–	+; l	–
P. virginiana L.	6.6 (3650)	–	–

Key: Plant parts: l = leaf; fl = flower; br = branch; fr = fruit; —: not investigated. Classification according to Schulze-Menz [21], but tribes and genera arranged alphabetically.

* In brackets: Voucher numbers (LEPS). 0.0 = not detected; <0.01 = trace amounts present.

† + = present; 0 = not detected.

‡ References in square brackets.

** Garden number.

floribundus) and 11.6% (*Sorbaria aitchisonii*) within the subfamily.

Turning to the Rosoideae, this subfamily is heterogeneous in sorbitol content. Thirty-three leaf samples were investigated which represented 33 species and all 8 tribes [21]. In agreement with Plouvier, sorbitol was lacking in Ulmarieae, Rubeae, Roseae, Sanguisorbeae and Potentilleae. Dryadeae proved to be heterogeneous; Geinae lacked sorbitol, whereas trace amounts of sorbitol were detected in Cercocarpinae and Dryadinae and small amounts in *Purshia tridentata* belonging to Purshiinae. By contrast, Kerrieae and Adenostomeae are sorbitol-rich taxa. *Coleogyne ramosissima* is a taxon of Rosaceae with a very high sorbitol content.

Twenty-two leaf samples were investigated in the Maloideae. Except for two samples of *Cydonia oblonga* (0.7 and 0.1%), all these taxa contained between 2.0% (*Malus silvestris*) and 8.7% (*Pyrus communis*) sorbitol. No clear-cut difference in sorbitol content exists between Maleae and Crataegeae. These results agree with those of Plouvier and other investigators.

All members of the Prunoideae previously investigated for the presence of sorbitol were shown to contain this hexitol. In the present investigation, the amounts of sorbitol present in leaves of 10 species varied between 0.3% (*Prunus laurocerasus*) and 16.8% (*Prinsepia sinensis*). The lowest contents were observed in leaves collected in summer from two evergreen shrubs, *Prunus laurocerasus* and *Prunus ilicifolia* (0.4%). The exceptionally high content of sorbitol in *Prinsepia sinensis* is in the very young leaves. These results are in full agreement with those of Plouvier and other investigators.

The sorbitol data clearly support the inclusion of *Lyonothamnus* in Spiraeoideae–Sorbaroeae and of the Ulmarieae in Rosoideae. Recent cytotaxonomic studies with *Filipendula* [22] likewise point to the rosoid affinities of Ulmarieae; all taxa and samples studied have $x = 7$. On the other hand Kerrieae with the genera *Rhodotypos*, *Kerria*, *Neviusia* and *Coleogyne* would fit better into the Spiraeoideae, on account of their high sorbitol content. The Kerrieae have been included in Spiraeoideae [23] or treated as a separate subfamily [24, 25]. Hegnauer [26] has pointed out that Kerrieae not only contain sorbitol, but also are cyanogenic and lack ellagic acid and are therefore out of place in the Rosoideae. Moreover, Bate-Smith [27] drew attention to the presence of the most unusual constituent leucopelargonidin in *Kerria japonica* and *Neviusia alabamensis* and stated that their inclusion in the subfamily Rosoideae is open to question. Chemically the Kerrieae are obviously similar to certain taxa of Spiraeoideae and Prunoideae. The same may be true of Adenostomeae, which according to the present observations contain sorbitol and are also known to be cyanogenic [28].

When the sorbitol data are compared with basic chromosome numbers (Table 2) some interesting features appear. Thus all Prunoideae have $x = 8$ and accumulate sorbitol, again all Maloideae have $x = 17$ and accumulate

sorbitol. The characteristic basic chromosome number of the Spiraeoideae seems to be $x = 9$, but $x = 8$ and $x = 10$ have also been reported. Moreover Goldblatt [29] showed that some genera deviate drastically in basic chromosome number (Table 2). This author has proposed including *Lindleya* and *Vauquelinia* (both not studied during this investigation) with Maloideae and the creation of a separate subfamily or tribe for *Quillaja* and *Kageneckia*. Exochordeae have $x = 8$; this monogeneric tribe was included by Juel [24, 25] and by Goldblatt [29] in Prunoideae. The presence of arbutin and of a number of flavones [30, 31] in *Exochorda* leaves, however, chemically links this genus more with certain Spiraeoideae (i.e. *Sorbaria*) or Maloideae (i.e. *Pyrus*) than with *Prunus*. The chromosome number $n = 27$ ($x = 9$; [29]) of *Lyonothamnus* agrees with its classification in Spiraeoideae [21].

The principal basic chromosome number of Rosoideae is 7. All members with this basic number lack sorbitol. The same is true of Alchemillinae which have the probably derived basic number 8. On the other hand Table 2 shows clearly that all taxa with $x = 9$ accumulate sorbitol (Kerrieae, Adenostomeae) or have at least small to trace amounts of sorbitol in their leaves (Dryadeae–Cercocarpinae, –Purshiinae, · Dryadinae). Dryadeae Geinae ($x = 7$) lack sorbitol. Kandler [32] recorded traces of sorbitol in leaves of species of *Rosa* and *Geum*. However, Kandler's results need confirmation, because his procedure of identification was not unambiguous.

The tribe Dryadeae is based on ovule number and structure [24, 25]. Karyological and chemical evidence suggest that this tribe is heterogeneous. Geinae evidently are true Rosoideae. The classification of the other subtribes remains open for the time being.

In higher plants sugar alcohols most probably have a storage function; they seem to replace the more usual storage carbohydrates in certain taxa [33, 34]. Hexitols, are more reduced than the corresponding hexoses; therefore, more chemical energy is stored in them. Other important functions of polyhydric alcohols in higher plants may rest in their involvement in different aspects of osmoregulation. Whatever the function of sorbitol may be in rosaceous plants, it is evident that sorbitol accumulation is an essential feature of many members of this family and that taxa showing this feature are ecologically diverse. Therefore the character does not express narrow ecological relationships between taxa, but rather may be an expression of relationship by descent.

EXPERIMENTAL

Reference compounds and other chemicals. Hexitols: D(−)-mannitol, Merck; D-sorbitol monohydrat, Flüka A. G.; dulcitol, BDH; monosaccharides: D-glucose, Merck; D(−)-fructose, Flüka

Table 2. Basic chromosome number and sorbitol content of dry leaves of Rosaceae*

Subfamilies	Tribes and subtribes	Basic chromosome number†	Sorbitol accumulation‡
Spiraeoideae	Exochordeae Exochorda	8	very strong
	-Lindleya	17§	–
	Gillenieae	9	very strong (Gillenia)
	Neillieae	9 (10)	very strong (Physocarpus)
	Quillajeae–Kageneckia	17§	–
	-Quillaja	14§	weak
	-Vauquelinia	15§	–
	Sorbarieae	9	medium to very strong
	Spiraeeae	9 (8, 7)	strong
Rosoideae	Adenostomeae	9	medium
	Dryadeae Cercocarpinae	9	trace amounts
	-Dryadinae	9	trace amounts (Dryas)
	-Geinae	7	0
	Purshiinae	9	weak (Purshia)
	Kerrieae	9	strong to very strong
	Pontentilleae-Alchemillinae	8	0
	-Potentillinae	7	0
	Roseae	7	0
	Rubeae	7	0
	Sanguisorbeae	7	0
	Ulmarieae	7 (8)	0
Maloideae		17	weak to very strong
Prunoideae		8	weak to very strong

* Classification of Schulze-Menz [21].

† In brackets, numbers also reported for the respective tribes.

‡ Not detected = 0; trace amounts only = <0.01 %; weak = 0.01–0.49 %; medium = 0.5–1.9 %; strong = 2–4.9 %; very strong = >5 %; not investigated = –.

§ According to Goldblatt [29].

A. G.; B.S.A.: *N,O*-Bis (trimethylsilyl)-acetamide (specially purified grade); DMF: dimethylformamide (silylation grade). GLC stationary phase: SE-30, Hewlett-Packard silicone rubber GE-SE30 50 GMS. Deactivator of inner glass surface of capillary columns: BTPPC = (Benzyl)-triphenyl-phosphonium chloride. Petroleum refers to fraction bp 40–60°; other solvents used were reagent grade.

Plant material. Only dried leaves were analyzed, taken from voucher specimens in the herbarium of the Laboratorium voor Experimentele Plantensystematiek (LEPS). Some leaf samples were collected from plants cultivated in the experimental garden or in the glasshouse of our laboratory (*Quillaja brasiliensis* Molina, *Purshia tridentata* DC., *Chamaebatiaria millefolium* Maxim.; see garden numbers in Table 1); in order to get strictly comparable results, these leaves were dried at 60° for 72 hr before extraction.

Extraction and purification. Leaves were ground in a ball mill and known amounts (usually 30–500 mg) were transferred quantitatively to a percolation apparatus as described by Hansen and Möller [17], which allows continuous extraction in the cold with a relatively small volume of solvent. Total extraction was achieved with 25 ml 80% aq. EtOH; this was checked [17]. The velocity of percolation was 1–2 ml/hr. The percolates were concentrated under red. pres. at 40° to 1–2 ml; the aq. residues were washed twice with 2–3 ml petrol. The purified aq. residues were adjusted to such a volume, that 1 ml corresponded to 30–50 mg of dried leaves.

Preparation of trimethylsilyl derivatives. One ml aliquots of the aq. extracts were transferred to small glass vials and dried under red. press. at 40° and then stored for at least 24 hr in a vacuum desiccator over P_2O_5. Silylation was carried out with equal vol. (50 μl) of DMF and BSA injected into the vial; the sample was allowed to react for at least 5 hr at 45°. Reference compounds (1–2 mg per vial) were treated in precisely the same way. Silylated samples remained stable for at least 2 days. In some instances 50 μl BSA were insufficient to achieve complete silylation; by increasing the amount of BSA to DMF to 3:2 it proved possible to get satisfactory results with such samples.

Gas-liquid chromatography. GLC analysis was carried out with a Perkin Elmer 3920B FID instrument and a BD8 Kipp en Zonen recorder; a Perkin Elmer M2 calculating integrator was used to give peak areas with baseline correction and retention times. Glass capillary columns were drawn and coated as described by Slob and Luteyn [35]. Columns varied in length from 25 to 30 m; int. ∅ was 0.3 mm and ext. ∅ was 0.7 mm. N_2 Carrier flow, 1.5 ml/min; on column injection was performed (0.1 μl); temperature of the injection block was 230°; the FID interface temperature was kept also at 230°. Different temperature programs were applied; the most commonly used programs were: (a) isotherm 220°; (b) 8 min isotherm 160°, consequently 2° per min until 220°, then isotherm at 220°; (c) 1 min isotherm at 80°, consequently 32° per min until 220°, next isotherm at 220°.

Identification and quantitative determination of sorbitol. The procedure described allows the clear discrimination of the isomeric hexitols sorbitol, dulcitol and mannitol. Supposed sorbitol peaks in plant extracts were identified definitively by comparing retention times with those of reference sorbitol peaks and by co-chromatography. In each instance, different programs were applied in order to exclude misidentifications. An approximate quantitative determination of the sorbitol content of leaf samples was achieved in the following way: every day 1–2 mg of sorbitol (exactly weighed) was silylated together with the leaf extracts to be analysed by GLC. The first and the last analysis of every day was performed with this sorbitol sample using the same temperature programs as for leaf extracts. By comparing the areas of the sorbitol peaks in the leaf extracts (derived from known amounts of leaf material) sorbitol percentages could be calculated. The variation coefficient (= standard deviation/mean × 100; n = 6) of this procedure was found to be less than 10%.

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