tative composition of these aliquots was determined by paper chromatographic procedures.

Inulobiose (1-β-fructofuranosyl-D-fructose) was disproportionated by transfructosidase to yield fructose and a new reducing fructosyl compound. Since the enzyme transfers fructose units from substrates to the 1-position of fructose moieties of cosubstrates,[5] it is probable that the synthesized compound is inulotriose, the trisaccharide of the inulin series.[7] Such a structure is also indicated by the identical R_f values obtained for inulotriose and for the synthesized compound.

Although stachyose did not function as a substrate for the enzyme, it did, nevertheless, partake in transfructosidation as a cosubstrate. An enzymatic digest of stachyose and sucrose contained the disproportionation products of sucrose as well as a new fructosyl compound. The new compound was non-reducing and moved on paper at a rate which would be expected for a pentasaccharide with a fructosylstachyose structure. Inclusion of C^{14}-sucrose in the digestion mixture resulted in the synthesis of radioactive fructosylstachyose. The radioactive compound was isolated, in pure form, and hydrolyzed to fructose and manninotriose. Activity measurements on the hydrolytic products showed that the fructose was the radioactive portion of the fructosylstachyose.

Planteose, the third oligosaccharide tested, did not function either as a substrate or cosubstrate for the transfructosidase. Apparently two substitutions on the fructose moiety of planteose eliminate it from the range of substrates or cosubstrates for the transferring enzyme of *Aspergillus oryzae*.

Experimental

Enzymatic Digests of Oligosaccharides.—A sample of 1 ml. of 0.04 *M* solution of the carbohydrate (inulobiose, planteose or stachyose) was mixed with 1 ml. of a 1% solution of enzyme concentrate.[10] Aliquots of 0.2 ml. were removed from the digest at time intervals of 0, 3, 6, 12 and 24 hours. The enzyme was destroyed by heat and the aliquots were examined for reaction products by multiple ascent paper chromatography.[5]

The compounds in the digest of inulobiose were resolved by two ascents of the solvent. The apparent R_f values[11] under these conditions were: 0.64 (fructose), 0.49 (inulobiose) and 0.36 (inulotriose). On prolonged enzyme action, the inulobiose was converted to fructose.

Some hydrolysis of the sucrosyl linkage in planteose and stachyose occurred after enzymolysis for 24 hours. There was, however, no evidence of transfructosidation in these digests. Under the same conditions new compounds were produced from sucrose and raffinose by the transferring enzyme.[5]

Enzymatic Digests of Mixtures of Oligosaccharides.—Digests of 0.5 ml. of 0.04 *M* sucrose solution, 0.5 ml. of 0.04 *M* solution of planteose, stachyose or inulobiose and 1 ml. of enzyme solution were prepared. Samples were obtained at time intervals of 0, 3, 6, 12 and 24 hours as above. Examination of these samples showed that disproportionation of sucrose had occurred, that planteose did not function as a cosubstrate, and that stachyose and inulobiose did function as cosubstrates. The apparent R_f values (6 ascents of solvent) of the reducing and non-reducing compounds in an enzymolysate of stachyose and sucrose were: 0.92 (fructose), 0.87 (glucose), 0.80 (sucrose), 0.61 (inulobiosylglucose), 0.48 (inulotriosylglucose), 0.22 (manninotriose), 0.15 (stachyose) and 0.07 (fructosylstachyose).[11] The R_f values (two ascents of solvent) of the reducing compounds in the digestion mixture of inulobiose and sucrose were identical with those listed in the preceding section.

Enzymatic Digest of C^{14}-Sucrose and Stachyose.—A sample of 2 mg. of C^{14}-sucrose (total activity *ca.* 6,000 c.p.m.) was dissolved in 0.1 ml. of a solution of sucrose (0.02 *M*) and stachyose (0.02 *M*). To this solution 0.1 ml. of the enzyme was added. The mixture was allowed to stand at room temperature for 18 hours. Two aliquots of 0.05 ml. of the digest were placed on a paper chromatogram. One-half of the developed chromatogram was sprayed with phloroglucinol reagent. On heating the sprayed chromatogram, the areas at which the fructosyl compounds are located appear as brown spots. These areas were cut from the chromatogram and their radioactivities determined in a conventional counting apparatus (Nuclear Scaler model no. 166). The activities of the compounds counted were as follows: fructose + glucose 792 c.p.m., sucrose 550 c.p.m., stachyose 2 c.p.m., and fructosylstachyose 144 c.p.m. The fructosylstachyose from the unsprayed portion of the chromatogram was extracted with water, taken to dryness, dissolved in 0.1 ml. of 0.05 *N* hydrochloric acid and heated at 80° for 30 minutes. The hydrolytic products (fructose and manninotriose) were separated on paper. The radioactivities of the products were found to be: fructose 132 c.p.m. and manninotriose 4 c.p.m.

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(10) The enzyme concentrate of *Aspergillus oryzae* was supplied by Takamine Laboratory, Inc., Clifton, N. J.

(11) Since multiple ascent chromatography was used, apparent R_f values are recorded. These values are obtained by dividing the height to which the individual compounds have moved by the total height of the paper strip. Differences in apparent R_f values of 0.05 are sufficient to give distinctly separated spots.

Reduction of Fluorine-containing Esters by Grignard Reagents

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It has been previously reported[1,2] that esters of perfluorinated acids could be reduced by Grignard reagents which contain β-hydrogen atoms to form the secondary alcohol corresponding to the alkyl group of the Grignard reagent. The explanation offered for this phenomenon is based on the ready reduction, demonstrated independently,[1,2] of the ketone formed as an intermediate in the reaction.

In this Laboratory, the reducing tendency of fluorine-containing esters has been employed as a means of preparation of secondary alcohols directly from the esters. By use of isopropylmagnesium halide as the reducing agent, it is possible to prepare secondary alcohols containing methyl, ethyl or phenyl groups from the corresponding Grignard reagents. The reactions are conducted employing a mixture of isopropyl and the alkyl or aryl Grignard desired. The experimental results are summarized in Table I.

It is interesting to note that as the length of fluorinated carbon chain of the ester increases the yield of reduction products is increased which is in accordance with previous observations in the perfluorinated aldehyde series.[1] No significant difference was observed between the reaction of a methyl or ethyl ester. Perhaps a more significant factor is the difference in reactivity observed between the isopropylmagnesium halide and the other Grignard reagents used with the former considerably less reactive under these conditions. This difference was not apparent when the reactions

(1) E. T. McBee, O. R. Pierce and J. F. Higgins, THIS JOURNAL, **74**, 1736 (1952).

(2) K. N. Campbell, J. O. Knobloch and B. K. Campbell, *ibid.*, **72**, 4380 (1950).

TABLE I

Ester	Grignard reagent	Product I	B.p., °C.	Yield, %	Product II	B.p., °C.	Yield, %
CF_3COOCH_3	Methyl + isopropyl	$CF_3CHOHCH_3$[a]	76–77	49			
$CF_3COOC_2H_5$	Methyl + isopropyl	$CF_3CHOHCH_3$[a]	76–77	60			
$C_2F_5COOCH_3$	Methyl + isopropyl	$C_2F_5CHOHCH_3$[b]	86–87.5	55	C_2F_5—$CHOHCH(CH_3)_2$[b]	108–109	10
$C_2F_5COOC_2H_5$	Methyl + isopropyl	$C_2F_5CHOHCH_3$[b]	86–87.5	55			
$C_3F_7COOCH_3$	Methyl + isopropyl	$C_3F_7CHOHCH_3$[c]	101–102	68	$C_3F_7CHOHCH(CH_3)_2$[g]	124–125	11
$C_3F_7COOC_2H_5$	Methyl + isopropyl	$C_3F_7CHOHCH_3$[c]	101–102	63			
CF_3COOCH_3	Ethyl + isopropyl	$CF_3CHOHC_2H_5$[d]	91–92	54	$CF_3CHOHCH(CH_3)_2$[h]	99–100	14
$C_2F_5COOCH_3$	Ethyl + isopropyl	$C_2F_5CHOHC_2H_5$[b]	99–100	48	$C_2F_5CHOHCH(CH_3)_2$[b]	108–109	17
$C_3F_7COOCH_3$	Ethyl + isopropyl	$C_3F_7CH(OH)C_2H_5$[e]	113–115	59	$C_3F_7CHOHCH(CH_3)_2$[g]	124–125	14
$C_3F_7COOCH_3$	Phenyl + isopropyl	$C_3F_7CHOHC_6H_5$[f]	94–95[i]	49	C_3F_7—$COH(C_6H_5)_2$[i]	147–148[i]	2

[a] F. Swarts, *Bull. soc. chim.*, **38**, 99 (1929). [b] E. T. McBee, J. F. Higgins and O. R. Pierce, THIS JOURNAL, **74**, 1387 (1952). [c] Calcd. for $C_5H_5F_7O$: C, 28.00; H, 2.34. Found: C, 28.02; H, 2.41. A small amount of $C_3F_7COH(CH_3)_2$ was formed. [d] See ref. 2. [e] E. T. McBee, O. R. Pierce and W. F. Marzluff, THIS JOURNAL, **75**, 1609 (1953). [f] Calcd. for $C_{10}H_7F_7O$: C, 43.50; H, 2.53. Found: C, 43.55; H, 2.58. [g] E. T. McBee, O. R. Pierce and M. C. Chen, THIS JOURNAL, **75**, 2324 (1953). [h] Calcd. for $C_5H_9F_3O$: C, 42.20; H, 6.32. Found: C, 42.11; H, 6.32. [i] Calcd. for $C_{16}H_{11}F_7O$: C, 55.00; H, 3.09. Found: C, 54.82; H, 3.14. [j] 10 mm.

were conducted with the individual Grignard reagent and may be attributed to the more bulky nature of the isopropyl group.

Experimental

A typical experiment is described as follows: A 2-liter, 3-necked flask was equipped with a mercury-sealed stirrer, an addition funnel, a reflux condenser and a calcium chloride drying tube. Six hundred ml. of anhydrous ether and 30.35 g. (1.25 moles) of magnesium turnings were placed in the reaction flask. A solution of 71 g. (0.5 mole) of methyl iodide, 92 g. (0.75 mole) of isopropyl bromide and an equal volume of ether was added slowly. After the addition was complete, the mixture was stirred for an additional hour. The Grignard reagent was cooled in an ice-bath and then a solution of 0.5 mole of the ester and an equal volume of anhydrous ether was added slowly. After the addition, the mixture was stirred overnight. The mixture was poured onto ice and then acidified with dilute hydrochloric acid. The ether was separated and the aqueous layer extracted several times with ether. The combined ether extracts were washed with several portions of a saturated sodium sulfite solution and then dried with anhydrous sodium sulfate. The ether solution was then rectified in a glass helix packed Todd distillation assembly.

The reaction products were identified by comparison of their physical properties to known compounds or, in the case of new materials, by analysis and infrared spectra determinations.

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Steroidal Sapogenins. X.[1] Qualitative Color Test for Pseudosapogenins

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The key step in transformation of steroidal sapogenins to pregnane derivatives is conversion to the so-called "pseudosapogenin" acetates,[2] *i.e.*, 20(22)-furostenol acetates. Because of the difficulty of crystallization of the products and the lack of significant detail in their infrared spectra, the extent of completion of the pseudomerization reaction may be followed, in a general way, by disappearance of the characteristic spiroketal absorption bands of the starting material,[3] rather than by the appearance of any properties characteristic of the product.

We have discovered that when the Tortelli–Jaffé color reaction[4,5] is applied to pseudosapogenins, a blue color is formed differing from the typical green color produced by ditertiary bridgehead ethylenic bonds or by olefins isomerizable to this type. The development of the blue color is apparently specific for the pseudosapogenin structure and does not occur with unsaturated steroids having double bonds at C_5–C_6, C_9–C_{11}, C_3–C_4, C_{16}–C_{17}, with dihydropseudosapogenins or with dihydrosapogenins. We have not tested pseudosapogenins with ditertiary, bridgehead ethylenic bonds.

The ultraviolet spectrum of the blue-colored material produced in the test shows maxima at 307 and 607 mμ (Beckman spectrophotometer). Bromine is transparent at 607 mμ at the concentrations used. Attempts to adapt the reaction for quantitative measurements were unsuccessful because the intensity of color developed reaches a maximum with about 1 mg. of steroid (in 10 ml. total volume) and is lower with quantities below and above this concentration. Color development does not follow the Beer–Lambert law. We have found the test to be particularly useful as an indication of completeness of reaction in pseudosapogenin transformations, *e.g.*, hydrogenation, oxidation, and the like.

Experimental

The color test was carried out in several ways. In the most sensitive method, the sample, 1 mg. of pseudosapogenin in 1 ml. of commercial C.P. chloroform, was diluted with 5 ml. of glacial acetic acid and mixed with 1 ml. of 0.1% bromine in chloroform. The mixture was underlayered with 0.1 ml. of 1% bromine in chloroform and after 30 minutes was diluted to 10 ml. with acetic acid and mixed.

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(2) R. E. Marker, *et al.*, see for example, THIS JOURNAL, **63**, 774 (1941); **69**, 2167 (1947).

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