Synthesis of D,L-α-Tocopheryl-α-D-mannopyranoside, a Potential Antiallergic and Antiinflammatory Compound, and its -α-D-mannofuranoside Isomer

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A new synthesis of D,L-α-tocopheryl-α-D-mannopyranoside, an antiallergic and antiinflammatory compound, has been accomplished. The synthesis was based on the following steps: the monosaccharide was stirred in a mixture of pyridine and acetic anhydride on an ice-water bath in order to obtain penta-O-aceyl-β-D-mannopyranoside. The protected mannopyranoside was dried exhaustively in vacuum on phosphorus pentoxide, mixed with D,L-α-tocopherol, dried again, and then glycosylation method according to Helferich was accomplished. Toluene constituted the reaction environment and p-toluenesulfonic acid was glycosylation promotor. Reaction mixture was submitted to Zemplen saponification and then to column chromatography on silica gel. Two mannosides have been separated in the molar ratio 40:1, the minor one migrating faster by thin layer and column chromatography. Being characterized by their ^{1}H and ^{13}C NMR spectra as well as by chemical and chromatographical means, the major mannoside proved to be D,L-α-tocopheryl-α-D-mannopyranoside and the minor one D,L-α-tocopheryl-β-D-mannofuranoside. They both could constitute new possible metabolites of α-tocopherol and mannose, as well as new substrates for α-mannosidases.

Key words: D,L-α-tocopheryl-α-D-mannopyranoside, D,L-α-tocopheryl-β-D-glucopyranoside, Helferich synthesis, glycosides; vitamins, ^{1}H and ^{13}C NMR spectra

In the last decades, α-tocopherol has been consecrated as being one of the most efficient antioxidant and radical scavenger [1, 2]. This remarcable biochemical and physiological function is due, at least partially, to the shielding of its phenolic group by hydrophobic methyl groups and its lateral chain [3]. The antioxidant and radical scavenger function of α-tocopherol is essentially dependent on the free state of its hydroxyl group. However, the use of α-tocopheryl glycosides makes sense and constant efforts have been made for their chemical and biochemical syntheses [4]. This phenomenon is due to the large widespread of hydrolytic enzymes cleaving their glycosides, produced either by mammalian host or the comensal microorganisms populating their digestive tract [5, 6].

Besides their free state, at least three types of natural compounds have been considered as important vitamins derivatives, both in structural and functional sense: carboxyl esters (both hydrophyllic and lipophyllic), phosphate esters and glycosides [7-9]. Due to their widespread distribution, vitamins glycosides were discovered relatively early, in comparison with their free state and the other two types of derivatives [9, 10]. In fact, their knowledge was tightly intertwined with and conditioned by the development of carbohydrate chemistry. A remarcable diversity of glycosides, either typical or esters, have been found in plants and microorganisms [9, 11].

In this paper, a new synthesis has been accomplished, of D,L-α-tocopheryl-α-D-mannopyranoside and -α-D-mannofuranoside and their ^{1}H and ^{13}C NMR spectra registered. According to our knowledge, D,L-α-tocopheryl-α-D-mannofuranoside is a new compound for the chemical and biochemical literature.

Experimental part

The following compounds or materials were purchased from Merck (Germany): α-tocopherol, D-mannose, sodium metal, pyridine, acetic anhydride, toluene, p-toluenesulfonic acid, silica gel for column chromatography, precoated glass plates with silica gel for thin layer chromatography (TLC). Penta-O-acetyl-β-D-mannopyranose has been synthesized according to [12], from dry D-mannose and an excess of pyridine/Ac_2O mixture, 2/1 (v/v). Glycosylation reactions were accomplished in dry toluene by using p-toluenesulfonic acid as chemical condensing agent [13]. Glycosylation mixture was diluted with chloroform while warm and filtered on a Celite pad. The filtrate was concentrated to dryness by rotavapor and the residue resumed in methanol and hydrolysed by Zemplen saponification with sodium methoxide. Column chromatography on silica gel followed, separation being monitored by TLC in solvent system (SS) I (chloroform-methanol-water, 50/10/1, v/v) [14, 15]. The purified glycosides, that proved homogenous by TLC were chemically and chromatographically analysed. A small portion of every mannoside was reacetylated and NMR spectra registered.

Analytical methods

Partial or total acidic hydrolysis and determination of chemical constituents were made as indicated (14-20).

NMR Spectra Registration

^{1}H And ^{13}C NMR spectra of all compunds, i. e., glycosylated D,L-α-tocopherol, D,L-α-tocopherol and carbohydrates, were registered in peracylated form in $CDCl_3$ containing TMS. Constantly, the spectra of peracetylated mannoside have been compared to the spectra of

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peracetylated aglycone and to the corresponding penta-O-aceyl-α-D-mannopyranoside. Moreover, we compared our spectra with similar spectra from literature: α-tocopheryl derivatives [21, 22], mannopyranosides [23-25], mannofuranosides [26]. NMR experiments were performed on a Bruker Avance DRX 400 spectrometer using 400 and 100 MHz for ^{1}H and ^{13}C frequencies, respectively. The ^{1}H-^{1}H correlation spectroscopy (COSY) and ^{1}H-^{13}C heteronuclear multiple quantum coherence (HMQC) experiments were carried out with an inverse probe.

D,L-α-Tocopheryl α-D-mannopyranoside. ^{1}H-NMR ($CDCl_3$; δ ppm; *J* Hz): 4.91 (bs, H-1'), 5.39 (H-2'), 5.62 (m, 2.8, H-3'), 4.51 (H-4'), 4.22 (H-5'), 4.34 (dd, 12.8, H-6'a), 4.18 (m, 10.8, H-6'b), as well as the signals characteristic to D,L-α-tocopherol [21, 22]: 0.84 (d, 3.6) 0.86 (d, 2.8); 0.87; 1.22 (s); 1.05-1.16 (m); 1.21-1.39 (m); 1.47-1.57 (m); 1.72-1.85 (m); 1.98 (s); 2.03 (s); 2.04 (s); 2.06 (s); 2.14 (s); 2.17 (s); 2.20 (s); 2.56 (t, 2.4).

^{13}C-NMR ($CDCl_3$; δ ppm): 101.54 (C-1'), 66.16 (C-2'), 69.10 (C-3'), 70.07 (C-4'), 68.17 (C-5'), 62.78 (C-6'), as well as signals peculiar to D,L-α-tocopherol [22]: 11.83, 12.09, 12.93, 19.62, 20.61, 21.04, 24.46, 31.06, 32.81, 37.32, 75.03, 117.33, 123.02, 124.88, 126.65, 128.33, 129.57, 140.57, 149.44, 169.65.

D,L-α-Tocopheryl-α-D-mannofuranoside. ^{1}H-NMR ($CDCl_3$; δ ppm; *J* Hz): 5.27 (d, 3.6, H-1), 5.20 (H-2), 5.61 (3.2, H-3), 4.33 (H-4), 5.25 (H-5), 4.67 (H-6a), 4.08 (H-6b).

^{13}C-NMR ($CDCl_3$; δ ppm): 108.13 (C-1), 77.03 (C-2), 76.18 (C-3), 77.35 (C-4), 69.96 (C-5), 62.79 (C-6).

Results and discussion

TLC Analysis of glycosylation mixture indicated the presence of two glycosides in different concentrations (fig. 1). They have been separated by column chromatography on silica gel where the heterogeneity of glycosylation mixture was confirmed (fig. 2). Silica gel column chromatography was repeated and homogenous fractions (fig. 3) were mixed and the mannosides analyzed. Both glycosides isolated by column chromatography contained D-mannose and D,L-α-tocopherol in the molar ratio 1:1. Compound migrating faster (the minor one) proved to be D,L-α-tocopheryl-α-D-mannofuranoside (fig. 4). Its chemical constituents indicated it to be a mannoside of D,L-α-tocopherol and its ^{1}H and ^{13}C NMR spectra disclosed its furanosic ring [14, 15, 19, 26]. The relative higher mobility of this compound, in comparison with D,L-α-tocopheryl-α-D-mannopyranoside, by TLC and column chromatography, confirmed a rule established previously: α-galactofuranosides of estrone, androstanolone, 11α-hydroxyprogesterone and prednisolone [19] as well as of cholesterol [27] migrated faster than the corresponding β-galactofuranosides. The major compound proved to be D,L-α-tocopheryl-α-D-mannopyranoside, according to its ^{1}H and ^{13}C NMR spectra [23-25]. A comparison between ^{13}C NMR spectra of D,L-α-tocopheryl acetate, D,L-α-tocopheryl-α-D-(tetra-O-acetyl) manno-pyranoside and penta-O-acetyl α-D-mannopyranoside has been made. A few remarks can be made: D,L-α-tocopheryl acetate presents practically no signal interfering with those of carbohydrates (between 60 and 110 ppm), while penta-O-acetyl α-D-mannopyranoside has no signal in the upperfield characteristic to D,L-α-tocopherol (except acetate groups). At the same time, C-1 of penta-O-acetyl α-D-mannopyranoside is represented by a signal at 90.43 (the other values being 62.07, 65.42, 68.20, 70.62, 73.29). C-1 of D,L-α-tocopheryl-α-D-(tetra-O-acetyl)manno-pyranoside has 101.54, while C-1 of D,L-α-tocopheryl-α-D-(tetra-O-acetyl)mannofuranoside is represented by a signal at 108.13, the amount obtained being too small to be further characterized.

Although the aim of this paper was the synthesis of D,L-α-tocopheryl-α-D-mannopyranoside, the corresponding -α-D-mannofuranoside have been also identified in the reaction mixture and partially characterized. In the course of the preparation of penta-O-acetyl α-D-manno-pyranoside, all precautions were taken to shun formation of the corresponding furanoside. Formation of the latter ring seems to be unavoidable, especially when are not used methods of synthesis that are rigorously specific either for pyranosic or furanosic ring. It was demonstrated [28] that even in special conditions, formation of mannopyranose is accompanied by about 3% mannofuranose. Our results confirmed this finding. Similar results were obtained in case of D-galactose: methods considered to produce exclusively pyranosic ring by acetonation were found to give rise also to furanose, although in traces. Such amounts could be identified either by MS [29] or by TLC [18].

The following vitamins have been found as glycosides in natural materials: pyridoxine, vitamin D, niacin, pantothenate, and riboflavin. Glycosylated forms of pyridoxine varies between 5 and 75% of the total vitamin B_6 content in fruits, vegetables and grains. The main glycosylated form of pyridoxine in most plant-derived foods is pyridoxine 5'-β-D-glucoside [30]. Ascorbic acid glucoside was prepared by incubating the aglycone with maltose or oligosaccharides and an enzyme produced by genera *Aspergillus* or *Penicillium* [31]. L-Ascorbic acid 2-glucoside that served for investigations of crystal structure and physico-chemical properties was produced by the action of the cyclomaltodextrin glucanotransferase (CGTase, EC 2.4.1.19) from *Bacillus stearothermophilus* [32]. Riboflavin glucoside was produced by cultivating a microorganism belonging to the genus *Bacillus* in a media containig the vitamin and starch [33]. Three glycosides of thiamin were prepared by using transferasic activities of the corresponding glycosidase: O-β-galactoside, O-α-glucoside, O--β-*N*-acetylglucosaminide [9, 34].

Although the exact location of vitamin E-metabolism has not been determined yet, it is known at molecular level: the lipid-soluble vitamin E is degraded to water-soluble carboxyethyl hydroxychroman metabolites by side-chain degradation without modification of the chromanol head. Carboxyethyl hydroxychroman metabolites are conjugated with glucuronic acid to increase their solubility and excreted in the urine [35]. A novel derivative of vitamin E, 2-(α-D-glucopyranosyl) methyl-2,5,7,8-tetramethyl-chroman-6-ol, was synthesized from 2-hydroxymethyl-2,5,7,8-tetramethylchroman-6-ol and maltose by transglycosylation with α-glucosidase from *Saccharomyces species*. The glycosylated product has relatively high solubility in water and its radical scavenging activity, measured with 1,1-diphenyl-2-picrylhydrazyl, was found to be nearly the same as those of α-tocopherol, 2-carboxy-2,5,7,8-tetramethylchroman-6-ol and ascorbic acid [36]. In this paper, direct mannosylation of D,L-α-tocopherol was made, the products being -α-mannopyranoside and -α-mannofuranoside. A number of hydroxysteroids have been galactofuranosylated, by using cadmium carbonate as promotor, as being representative in terms of type of hybridization of carbon bearing hydroxyl group, position on cyclopentenoperhydrophenantrene nucleus and, implicitly, the degree of shielding: cholesterol, estrone, androstanolone, 11-α-hydroxyprogesterone, prednisolone [19]. Glycosylation of cholesterol produced cholesteryl β-D-galactofuranoside and -α-D-galacto-furanoside in the ratio 10:1 [14, 19, 27]. The major product of galactofuranosylation of the other hydroxysteroids was

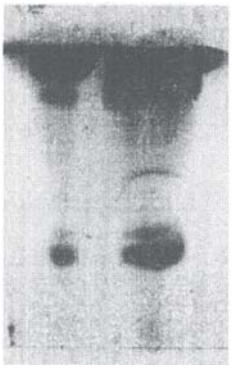


Fig. 1. TLC Analysis of the total glycosylation mixture. Migration SS I, visualization, mostain

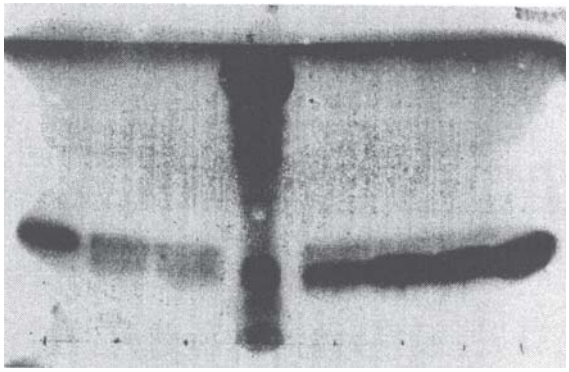


Fig. 2. TLC Analysis of fractions from silica gel column chromatography separation of D,L--α-tocopheryl mannosides. Migration SS I, visualization, mostain

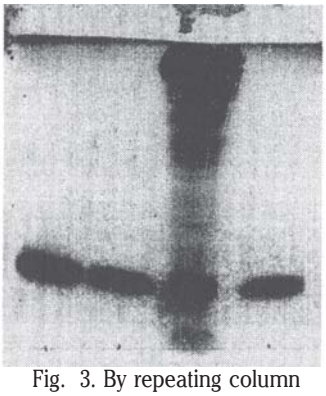
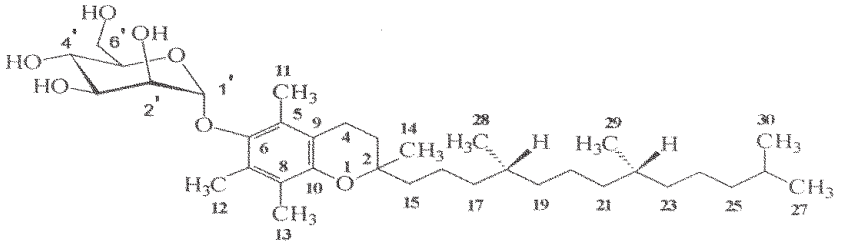
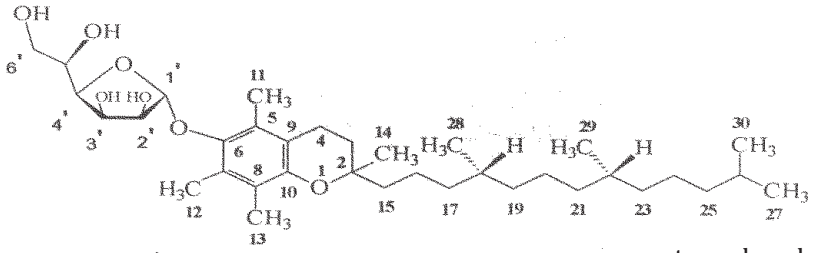


Fig. 3. By repeating column chromatography, pure compounds have been obtained. Migration SS I, visualization, mostain



D,L - α - Tocopheryl - β - D - mannopyranoside



D,L - α - Tocopheryl - β - D - mannofuranoside

Fig. 4. Comparative structure of the synthesized compounds: D,L-α-tocopheryl--α-D-mannopyranoside and D,L-α-tocopheryl -α-D-mannofuranoside

also the corresponding β-D-galactofuranoside [19]. Two furanosylated glycosphingolipids – β-D-galactofuranosyl-6- and β-D-galactofuranosyl-3-α-D-galactopyranosyl-1'ceramide – have been synthesized by using galactocerebroside and sulfatide, respectively, as precursors [37]. In all these reactions, both anomers were produced, the β anomer being the major one. All these results, as well as the results of the present paper, constitute a confirmation of a previous theory concerning glycosides synthesis, maintaining that 1,2-*trans*-glycosidic linkages are favoured [38].

DL-α-Tocopheryl-α-D-mannopyranoside has remarcable properties due to its constituents: a strong affinity to biomembranes through interaction of its side chains with arachidonic acid in the phospholipids of the membrane [39-41] and the capacity to alter the linkage of immunoglobulins (IgG, IgM, IgE, IgA, IgD) to different ligands, due to its carbohydrate moiety. It has been demonstrated, in a detailed and complex experiment, that DL-α-Tocopheryl-α-D-mannopyranoside has excellent anti-allergic and anti-inflammatory activities. At least the first one was attributed to an interpherence of the mannoside in the linkage between antigen and IgE, due to the fact that the sugar chain attached to Fc fragment of the δ-chain in IgE is mainly mannose [42]. DL-α-Tocopheryl-α-D-mannofuranoside could offer a strategy to enhance this interpherence.

Conclusions

Peracetylation of D-mannose in the cold and use of the protected sugar in Helferich glycosylation of DL-α-tocopherol, with p-toluenesulfonic acid as promotor, produced a mixture of DL-α-tocopheryl-α-D-mannopyranoside and -α-D-mannofuranoside.

Although in trace ammounts, DL-α-Tocopheryl-α-D-mannofuranoside produced could be isolated and partially characterized.

^{1}H and ^{13}C NMR Spectroscopy of aglycone, glycosylation donor and glycoside proved a useful instrument in monitoring the glycosylation reaction as well as in products characterization.

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Manuscript received: 19.02.2009