Two Structural Alternatives of Glycosides of Vitamins: Pyranosic and Furanosic Ring of Sugar

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Abstract

A new compound has been synthesized, D,L-α-tocopheryl-β-D-galactofuranoside, as a new possible metabolite and substrate for β-galactofuranosidases. The synthesis included the following steps: the monosaccharide was heated in pyridine and then peracylated while still warm. Furanosic products, i. e., penta-O-benzoyl-αβ-D-galactofuranosides, have been separated from the pyranosic ones by fractional crystallization and characterized by chemical and physical methods. Bromination of the galactofuranosides mixture with hydrobromic acid produced the glycosylation donor, tetra-O-benzoyl-α-D-galactofuranosyl bromide. Koenigs-Knorr D-galactofuranosylation of D,L-α-tocopherol, by using cadmium carbonate as promotor, produced D,L-α-tocopheryl-β-D-(2,3,5,6-tetra-O-benzoyl)galactofuranoside. Protecting groups were removed by Zemplen saponification and the galactofuranoside purified by column chromatography on silicagel. The new synthesized compound was characterized by physical-chemical and chromatographical means.

Keywords: D,L-α-tocopheryl-β-D-galactofuranoside; Koenigs-Knorr synthesis; glycosides; vitamins

Introduction

Besides their free state, at least three types of natural compounds have been consecrated as important vitamins derivatives, both in structural and functional sense: carboxyl esters (both hydrophyllic and lipophyllic), phosphate esters and glycosides [1] [2]. Due to their widespread distribution, vitamins glycosides were discovered relatively early, in comparison with their free state and the other two types of derivatives [3]. In fact, their knowledge was tightly intertwined with and conditioned by the development of carbohydrate chemistry. A remarkable diversity of glycosides, either typical or esters, have been found in plants and microorganisms [4].

Five water-soluble glycosides have been found as pigments of safron (*Croccus sativus*): crocin, the main one (about 80 %), is β,β'-digentiobiosyl 8,8'-diapocarotene-8,8'-oate, and the other four, β,β'-D-diglucosyl 8,8'-diapocarotene-8,8'-oate; β-D-gentiobiosyl β-D-glucosyl 8,8'-diapocarotene-8,8'-oate; β-D-glucosyl hydrogen 8,8'-diapocarotene-8,8'-oate; β-D-gentiobiosyl hydrogen 8,8'-diapocarotene-8,8'-oate. These structures were confirmed by spectral means: ^{1}H NMR, UV-visible, IR and MS [5]. Concomitantly, another glycoside was found in safron, picrocrocin, i. e., 3-hydroxy-β-cyclocitral-β-D-glucoside [6]. Subsequently, by using (–)-3-methoxy-β-ionone, it was proved that chiral center of picrocrocin aglycon is identical with the two chiral centers of zeaxanthin, namely the R-configuration [7]. This finding indicated metabolic relationships between these glycosides [6]. A partial

hydrogenated glycoside, in the carotenoid aglycon, was found in *Streptococcus facium*, a nonphotosynthetic bacterium, its structure being 4-D-glucopyranosyloxy-4,4'-diapo-7,8-dihydro-ψ,ψ-carotene [8] [9]. Glycosides having C_{40}-carotenoids based on D-glucose or L-rhamnose have been isolated from myxobacteria. Interestingly, glucosides are esterified with a fatty acid while rhamnosides are not, both types of glycosides having the glycosidic bond on a tertiary hydroxy group of aglycon [10] [11]. Two non-allylic glycosides based on L-rhamnose and zeaxanthin have been also completely characterized: [3R,3'R]-3'-α-L-rhamnosyloxy-β,β-carotene-3-ol and [3R,3'R]-3, 3'-α-L-dirhamnosyloxy-β,β-carotene [12]. A first conclusion could be drawn from these experiments: C-40 and C-50 carotenoid glycosides are based expecially on D-glucose and L-rhamnose.

The glycosidic moiety can play a crucial role for the activity and improving pharmacokinetic parameters. At the same time, due to recent progress in molecular glycobiology, the comparative biological actvities of aglycone *vs*. glycoside became more clear and it became possible to imagine and synthesize glycodrugs characterized by their specificity and efectiveness. In general, sugar moieties increase polarity, and implicitly the hydrophilic character of glycoside, in comparison with the free aglycons. In this way, their pharmacokinetic properties – circulation, concentration, remanence, concentration variation – are improved. Glycosylation strongly alter the transport through physiological barriers, e. g., brain barrier, placental barrier. Remarcably, some glucosides can be actively carried into the brain tissue by using the glucose-transport system. Contrary, many glucuronides are blocked by placental barrier, thus preventing intoxication of foetal tissue by conjugates of xenobiotics [13]. Another aspect is the interaction between some glycosidic fragment and the respective complementary zone of receptors or lectins. A good example is the high affinity of β-galactosides to hepatocytes due to galectin-C occurring in high concentrations on cell surface [14]. Cleavage of glycosidic bond, along the gastrointestinal tract or in the blood circuit or in the synovial fluid of joints [13] [15], is another important facet of biochemical and physiological actions of glycosides. Some glycosides are cleaved by acidic environment of the stomach, others pass unaffected by this region and are hydrolyzed by glycosidases of small intestine and the third group reach the colon and are metabolized by the intestinal microflora [13].

In this paper, a new derivative of α-tocopherol has been synthesized, separated and characterized, i. e., D,L-α-tocopheryl-β-D-galactofuranoside. It could be a new metabolite of α-tocopherol or serve as an enzymatic substrate of β-D-galactofuranosidases or as galactofuranosyl donor in the transferasic reactions of hydrolases.

Materials and methods

Materials. D,L-α-tocopherol, D-galactose, benzoylchloride, toluene, cadmium carbonate, sodium metal, acetic anhydride, pyridine, ethanol, thin layer plates ready-to-use, silicagel for column chromatography, methanol, chloroform, 1,2-dichloroethane, calcium sulfate, concd. sulfuric acid, ammonium molibdate, $Ce(SO_4)_2$, were either from Merck or from Fluka.

Methods used

Infrared (IR) spectra were registered in KBr pellet or as a solution in Nujol. IR Spectra of free and peracylated galactofuranoside were compared with the corresponding spectra of D,L-α-tocopherol and acylated D,L-α-tocopherol, respectively.

Acetylation. Two compounds were submitted to acetylation in this paper, D,L-α-tocopherol and D,L-α-tocopheryl-β-D-galactofuranoside. Alternatively, the two compounds were solved in pyridine and stirred overnight with an excess of acetic anhydride, the ratio between acetic anhydride and pyridine being 2/1 (v/v).

Benzoylation. The two compounds mentioned above, i. e., D,L-α-tocopherol and D,L-α-tocopheryl-β-D-galactofuranoside were benzoylated. The respective compound was solved in pyridine, the solution was cooled on ice and benzoyl chloride was added in small portions, the ratio between solvent and benzoylatig agent being 10:1. Then pyridine was removed by coevaporating with toluene. Excess benzoyl chloride was decomposed by adding ice in the reaction mixture and the acidic products formed (HCl, benzoic acid) were removed by partition between chloroform and a saturated solution of $NaHCO_3$.

Glycosylation agent and Koenigs-Knorr glycosylation. Penta-O-benzoyl-αß-D-galactofuranoside was prepared as indicated by D'Accorso et al [16]. By bromination of perbenzoylated sugars with hydrobromic acid in glacial acetic acid, exclusively 1-bromo-1-deoxy-α-D-2,3,5,6-tetrabenzoyl-galactofuranoside was obtained [17]. Glycosylation of D,L-α-tocopherol was performed in boiling dry toluene, by using cadmium carbonate as chemical condensing agent [18]. Freshly dried calcium sulfate was used as water scavenger. Thin layer chromatography (TLC) was achieved on ready-to-use plates in the following mixtures: solvent system (SS) I (toluene-n-hexane, 1/1, v/v), SS II (toluene-methanol, 7/1, v/v), SS III (toluene-ethanol, 5/1), SS IV (chloroform-methanol-water, 50/10/1, v/v), SS V (chloroform-methanol-water-concentrated ammonia, 70/30/4/1, v/v). Visualisation was made by dipping the plates in a solution called mostain consisting of water, sulfuric acid, ammonium molybdate and Ce $(SO_4)_2$, followed by heating on a hot plate. Because reaction products interphere with initial reactants by TLC, the following general strategy was adopted: reaction mixture was filtered on Celite, the filtrate was evaporated to dryness in vacuum, the residue mixed with sodium methoxide in methanol (Zemplen saponification) and stirred till alkaline hydrolysis attained the completion (TLC, SS IV). Reaction mixture was then neutralized, concentrated to dryness and the residue submitted to repeated Folch partition [19]. Chloroformic phase was retained and the product was chromatographed on a column of silica gel, elution being made with an increasing gradient of chloroform in methanol. Fractions were monitored by thin layer chromatography in SS IV. In this way, unreacted 1-bromo-derivative as well as the accidentally produced galactosyl-ß-1-1'-ß-galactoside were removed in aqueous phase. The main reaction product was regained, together with unreacted D,L-α-tocopherol, in chloroformic phase. The latter two compounds have been clearly separated by column chromatography on silica gel and the respective glycolipid was submitted to physical-chemical analyses or, alternatively, peracetylated or perbenzoylated. D,L-α-Tocopherol and D-galactose of tocopheryl galactoside were colorimetrically determined after acidic hydrolysis and partition: D,L-α-tocopherol by Emmerie-Engel [20] and D-galactose by anthrone reaction [21].

Results and discussion

D,L-α-Tocopherol, D,L-α-tocopheryl acetate and D,L-α-tocopheryl benzoate constituted reference compounds for spectral, chromatographical and physico-chemical properties of the corresponding D-galactofuranosic glycoside, either in free or peracylated form. Acetylation of D,L-α-tocopherol was followed in two SS (Fig. 1). In fact, D,L-α-tocopheryl acetate is hydrolyzed by a very specific enzyme isolated from chicken liver microsomes. The enzyme could have a role in D-α-tocopherol metabolism [22].

Galactofuranosylation reaction could be followed by TLC of reaction products: both compounds migrating faster and slower than D,L-α-tocopherol could be noticed, respectively (Fig. 2). More evident yet that glycosylation reaction had been accomplished, appeared after Zemplen saponification of total reaction products (Fig. 3).

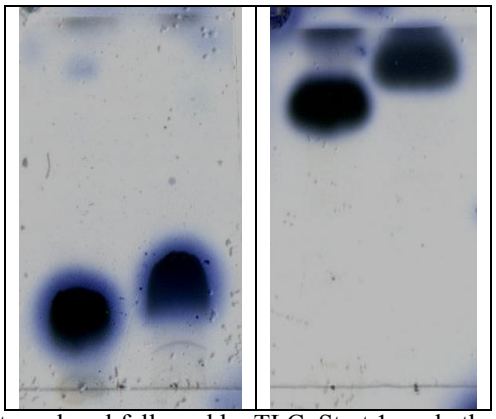


Figure 1. Acetylation of D,L-α-tocopherol followed by TLC. Start 1, on both plates, D,L-α-tocopherol; start 2, on both plates, D,L-α-tocopheryl acetate. Migration: plate one, SS I; plate two, SS II. Visualisation, mostain.

Column chromatography on silicagel (Fig. 4) led to the isolation of D-galactofuranoside. Beside chromatographic mobility, typical for a lipidic glycoside, the increasing of chromatographic mobility by acetylation [23], comparable with cerebrosides or sterayl monoglycoside, constitutes another proof for a glycoside. Hydrolysis of the isolated galactofuranoside (Fig. 6) produced D,L-α-tocopherol and D-galactoside in the molar ratio 1:1 (Fig. 7).

Figure 2. TLC Analysis of D-galactofuranosylation products of D,L-α-tocopherol. Start 1, D,L-α-tocopherol; start 2, reaction products. Migration, SS II. Visualisation, mostain.

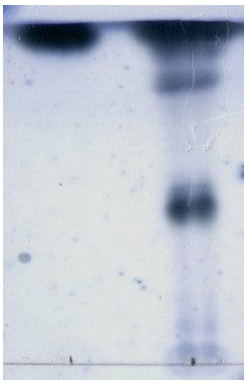


Figure 3. TLC Identification of D-galactofuranosylation product after Zemplen saponification. Start 1, crude reaction mixture; start 2, reaction mixture after Zemplen saponification. Migration, SS IV; vizualization, mostain.

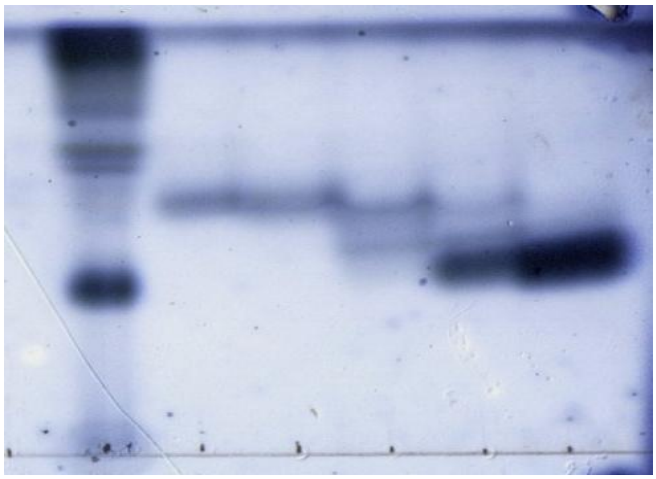


Figure 4. TLCmonitoring of D,L-α-tocopheryl-β-D-galactofuranoside separation by column chromatography on silicagel. Start 1, total mixture; starts containing separated fractions. Migration, SS IV; vizualization, mostain.

Infrared spectra of peracylated galactofuranoside, in comparison with the native compound, indicated the dissapearance of the absorbance characterstic to hydroxy groups (3500 cm^{-1}). Another important aspect was the fact that by benzoylation of native tocopheryl galactofuranoside, a reaction product was reconstituted.

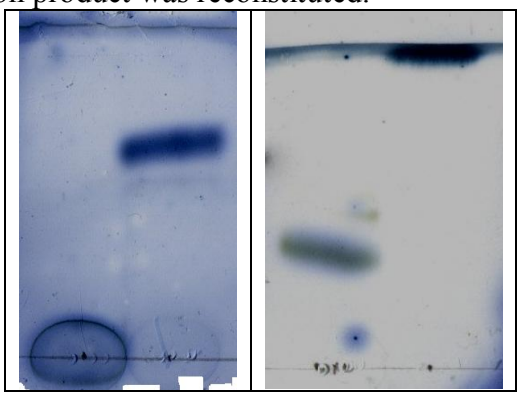


Figure 5. Peracetylation of tocopheryl glycoside. Start 1, on both plates, D,L-α-tocopheryl-β-D-galactofuranoside; start 2, on both plates, peracetylated product. Migration, plate one, SS II, plate two – SS IV. Visualisation, mostain.

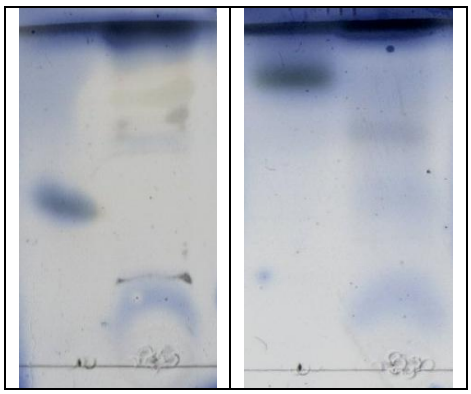
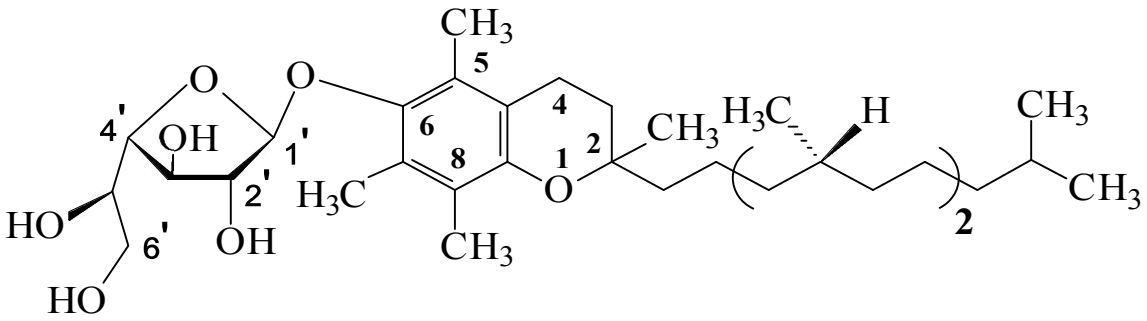


Figure 6. Acidic hydrolysis of tocopheryl glycoside. Start 1, on both plates, D,L-α-tocopheryl-β-D-galactofuranoside; start 2, on both plates, hydrolysis product. Migration, plate one, SS IV, plate two – SS V. Visualisation, mostain.



D,L - α - Tocopheryl - β - D - galactofuranoside

Figure 7. D,L-α-tocopheryl-β-D-galactofuranoside, a new possible metabolite of D-α-tocopherol and D-galactose.

Although vitamins are micromolecules, and they do not present the structural complexity of macromolecules, they raise special problems for glycosylation reaction. Some of them are thermolabile (vitamin B1, aneurine), others are photosensitive (vitamin B2, riboflavin; vitamin A, retinol) and almost all of them are extremely susceptible to oxidation, vitamin F (polyunsaturated fatty acids) inclusively. Moreover, hydroxyl group envisaged to be glycosylated is shielded by other functional groups [1] [2]. These aspects have been circumvented in two ways: (A) the elaboration of glycosylation methods running at relatively low temperatures (room temperature, 0^0C, – 30^0C or even lower); (B) the use of biochemical methods, in fact the transferasic activities of hydrolytic enzymes.

As a happy coincidence, some chemical glycosylation methods are favored by low temperatures. Formation of a series of C_7-C_{16}-alkyl D-glucopyranosides from penta-O-acetyl β-D-glucopyranoside and the corresponding alcohol in the presence of $SnCl_4$ was induced at room temperature [24]. 2-Hydroxyethyl 2',3',4',6'-Tetra-O-benzoyl-β-D-galactopyranoside was synthesized in acetonitrile, molecular sieves and tetra-O-benzoyl α-D-galactopyranosyl bromide in CH_2Cl_2 and a solution of silvertriflate and 2,6-ditert-butylpyridine in toluene at temperatures of – 30 – +20 ^{0}C [25]. It was proved that acetobromoglucose reacted with C_6-C_{12} alcohols (even-numbered, normal chain) at room temperature in the presence of Ag_2O [26]. In order to synthesize α-L-fucopyranosyl-2-lactose, at room temperature, the protected L-fucose was activated as 1-thiomethyl and promoter was iodonium di(sym-collidine) perchlorate [27]. Glycosylation of tigogenin with α-acetobromoglucose, by using silver 4-hydroxyvalerate as promotor, gave a yield of 55 % at room temperature and 65 % at – 10 ^{0}C in diethyl ether [28]. One of the numerous synthesis of trehalose was made by stirring α-D-acetobromoglucose and 2,3,4,6-tetra-O-acetyl-β-D-glucose in the presence of mercuric cyanide at room temperature [29]. A similar strategy was adopted for D-galactofuranosylation of vitamin B1 (aneurine): tetra-O-benzoyl α-D-galactofuranosyl bromide was stirred for 72 hrs at room temperature in a suspension containing toluene, D,L-α-tocopherol, $CdCO_3$, $CaSO_4$ (D. P. Iga, unpublished data). Concerning glycosylation of tocopherol, this compound is relatively resistant to heating. β-D-Galactopyranosylation of tocopherol was made by heating the sugar (D-glucose, D-galactose, D-manose) as penta-O-acetate pyranoside with D,L-α-tocopherol, or D,L-δ-tocopherol, and catalytic amounts of p-toluenesulfonic acid at 90 °C for 5 h in vacuum [30]. In order to avoid blockage of phenolic group of tocopherol, lateral chain was replaced by a hydroxymethyl group to which α-D-glucopyranosyl group was linked as a glucoside [31].

L-Ascorbic acid glucoside, in stable form, was produced by the action of the cyclomaltodextrin glucanotransferase (EC 2.4.1.19) from *Bacillus stearothermophilus* and crystallized from an aqueous solution. Determination of the molecular structure by single crystal X-ray analysis showed the compound to be 2-O-α-D-glucopyranosyl-L-ascorbic acid [32]. 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 [33]. Ascorbic acid glucoside was prepared by incubating the aglycone with maltose or oligosaccharides and an enzyme produced by genera *Aspergillus* or *Penicillium* [34]. Riboflavin glucoside was produced by cultivating a microorganism belonging to the genus *Bacillus* in a media containig the vitamin and starch [35]. Three glycosides of thiamin were prepared by using transferasic activities of the corresponding glycosidase: O-β-galactoside, O- α-glucoside, O-β-*N*acetylglucosaminide [13] [36].

Conclusions

1.Glycosylation of D,L-α-tocopherol with tetra-O-benzoyl-α-D-galactofuranosyl bromide, by using cadmium carbonate as promotor, produced D,L-α-tocopheryl-β-D-(2,3,5,6-tetra-O-benzoyl)galactofuranoside.
2.Zemplen saponification of reaction products produced D,L-α-tocopheryl-β-D-galactofuranoside, as became evident by its constituents (D,L-α-tocopherol and D-galactose) in the molar ratio 1:1, comparative chromatographical behaviour before and after acylation, IR spectroscopy.
3.Reaction product, as glycoside, although relatively susceptible to acidic treatement, is stable in view of biochemical or biological experiments.

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