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Synthesis and cytotoxic activity of 4-O-β-D-galactopyranosyl derivatives of phenolic acids esters

Nader Al Bujuq[a] [ID], Sharif Arar[b] and Raida Khalil[c]

[a]Chemistry Department, Deanship of Academic Services, Taibah University, Medina, Kingdom of Saudi Arabia; [b]School of Science, Department of Chemistry, The University of Jordan, Amman, Jordan; [c]Department of Biotechnology and Genetic Engineering, Philadelphia University, Amman, Jordan

ABSTRACT

The glycosylation of naturally occurring phenolic acids has a significant impact on their solubility, stability and physiochemical properties. D-Galactose residue was found to form a part of glycoconjugates in several tissues and involved in a variety of physiological process. To the best of our knowledge, we have noticed a little information about the glycosylation of the phenolic acids with galactose residue. In this work, we describe the glycosylation of methyl vanillate and methyl ferulate with peracetylated-β-D-galactopyranose in the presence of $BF_3 \cdot OEt_2$. The coupling reaction yielded efficiently and selectively only the acetylated β-D-galactopyranosides **3** and **6**. Removal of the acetyl groups using sodium methoxide afforded the corresponding β-D-galactopyranosides **4** and **7** in good yields. Anticancer activity *in vitro* was evaluated against two human cancer cell lines (MCF-7 breast cancer cell lines and PC-3 prostate cancer cell lines). β-D-galactopyranosides **4** and **7** demonstrated improved cytotoxic activity compared to the parental esters.
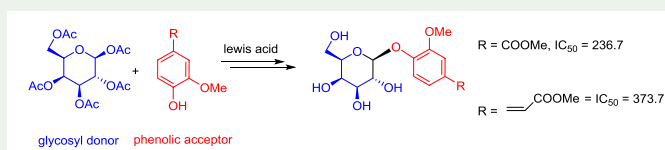
Abbreviations: FE   Ferulic acid methyl ester (**2**); VE   Vanillic acid methyl ester (**5**); Gal-FE   β-galactopyranosyl ferulic acid methyl ester (**4**); Gal-VE   β-galactopyranosyl vanillic acid methyl ester (**7**)

1. Introduction

Phenolic acids are natural products compounds that are found in many types of plants (Aruoma et al. 2005) their constituents are considered very important of the human diet due to their benefit antioxidant activity (Martin and Appel 2010). Recent interest in phenolic acids comes from their potential protective role, through ingestion of fruits and vegetables,

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CONTACT Nader Al Bujuq ✉ nbujuq@yahoo.com, nbujuq@taibahu.edu.sa

Ⓢ Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1375927.

against oxidative damage diseases (coronary heart disease, stroke) (Bravo 1998), also their potentially important properties such as anticancer activities (Liu 2004; Harris et al. 2007; Huang et al. 2010). Wide varieties of phenolic acids exhibit various physiological activities, including antibacterial, anti-inflammatory and anticarcinogenic (Nohynek et al. 2006; Russell and Duthie 2011). Researches about biological and pharmacological activities have also been documented for phenolic compounds, including free radicals scavenging, apoptosis of cancer cells (Kanai and Okano 1998; Saeki et al. 2000).

The glycosylation of phenolic compounds is considered a vital method due to increase their solubility and stability in water (Plaza et al. 2014; De Winter et al. 2015). Glycosylation of phenolic groups protect the hydroxy groups from oxidation (Torres et al. 2011; Zhang et al. 2013). Also, glycosylation can potentially improve pharmacokinetics, pharmacodynamics, solubility, mechanism and potency.

Variation of the introduced glycosyl residues shows different impact on the properties of phenolic compounds. The galactosylated hydroquinone is 1.19 times higher antioxidant than the glucoside counterpart β-arbutin (Kim et al. 2010). More interestingly, there exist abundant galactose acceptors on the hepatocyte surface that could bind with galactose derivatives (Mishra et al. 2015). Thus, the glycosylation with galactose could aid targeted delivery of drugs to liver cancer cells but not to nearby normal cells (Gankhuyag et al. 2015). Moreover, galactose residue forms part of glycolipids and glycoproteins in several tissues and considered to be involved in a variety of biological recognition events, the synthesis of galactosyl phenolic derivatives would be of scientific and practical interest (Ajisaka and Yoon 1996). To the best of our knowledge, no information was reported about the glycosylation of the phenolic acids with certain sugars such as galactose. Most of these procedures were multisteps, tedious and cumbersome, besides the compounds were not fully characterised (Ding et al. 1996; Lu et al. 2015; Fei et al. 2016). In this paper, we described a simplified and straightforward synthetic route of methyl ferulate and methyl vanillate galactopyranosides **4** and **7** under mild conditions. β-D-galactose pentaacetate **1** was utilised as a glycosyl donor, while the methyl esters **2** and **5** were used as acceptors. As a result of glycosylation, the novel phenolic β-D-galactopyranosides **4** and **7** were produced selectively in good yield. In this paper, we also aimed to investigate the cytotoxic activity of the novel phenolic β-D-galactopyranosides **4** and **7** against two human cancer cell lines (PC-3 prostate cancer cell lines and MCF-7 breast cancer cell lines). The results of the glycosides **4** and **7** showed superior cytotoxicity effect on the cell lines compared with its corresponding free ester.

2. Results and discussion

2.1. Synthesis

Currently, phenolic glycosides are available through three different approaches: extraction from natural sources, chemical synthesis and enzymatic synthesis. Extraction of phenolic glycosides from natural sources is complex and uneconomical as the target compound in natural organisms is usually of low content. In contrast, synthetic methods including chemical synthesis and enzymatic synthesis are efficient and can produce either natural or unnatural phenolic glycosides with high values (Sears and Wong 2001; Yu et al. 2012). As a consequence, the synthesis methods have received increasing attention for developing active phenolic glycosides.

The stereoselective formation of anomeric centres is a major problem in the synthesis of glycosides. Many combinations of leaving group, promoter, and protecting group have been devised to achieve this. To overcome the formation of mixture of anomers, we started the synthesis using β anomer of the D-galactose pentaacetate **1**. Nevertheless, $BF_3.OEt_2$ is a powerful promoter for the activation of acetyl groups, it was found to be effective for the coupling between the glycosyl donor **1** and the phenolic acids derivatives acceptors **2** and **5**. These couplings were proven to be feasible, and the corresponding glycosides **3** and **6** were produced selectively with only β anomers.

Generally, it is considered that the formation of **3** and **6** follows an SN_1-like mechanism. Practically the glycosylation reaction begins by the interaction taking place between promoter ($BF_3 \cdot OEt_2$) and the glycosyl donor **1**. This results in the formation of an activated species followed by the dissociation of a leaving group. The reaction may proceed via the formation of glycosyl cation that can be stabilised via an acyloxonium intermediate which can be formed as a result of the participation from the carbonyl group at C-2. These intermediates can be glycosylated with the glycosyl acceptor (ROH). Due to the presence of acetyl group at C-2 (anchimeric assistance), (ROH) attacks the dioxolenium ion predominantly from one face to provide a 1,2-trans glycoside which explains the formation of β-glycosides selectively.

The optimisation condition of the reaction to give high yield and high selectivity of the β-glycoside was carried out in Dichloromethane at 0 °C for 8 h. The preliminary investigation of ^{1}H NMR sample of the acetylated intermediate confirmed the formation of β anomer selectively without any formation of α anomer. The prolonged time above 0 °C led to anomerisation. When the reaction was done at higher temperatures, a series of mixture products were obtained and decomposition appeared. Other lewis acids, for instance, TMSOTf, NIS/TfOH were employed to improve the yield. However, the selectivity and the efficiency were not adequate (Table 1).

The identification of compound **3** was readily confirmed on the basis of its, ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR showed two singlet of methyl signals at 3.80 and 3.73 ppm representing (–O*Me*) and (–COO*Me*), respectively. The anomeric proton appeared at 5.48 ppm as doublet with $J = 9.0$ Hz, the chemical shift and the value of the coupling constant confirm the formation of only β anomer stereoselectively. The sugar protons appeared at the normal position range (3.92–4.21 ppm). Three aromatic protons appeared at 7.25–7.64 ppm. The

**Table 1.** Glycosylation of **1** and **2** in the presence of various acidic catalysts at different reactions conditions.

Entry	Glycosyl donor	Ferulic acid methyl ester	Reaction Time	Temperature (°C)	Acid catalyst promoter	β/α	% of starting material
1	1	2	15 min	0	$BF_3 \cdot OEt_2$	Only β	85
2	1	2	60 min	0	$BF_3 \cdot OEt_2$	Only β	34
3	1	2	4 h	0	$BF_3 \cdot OEt_2$	Only β	22
4[a]	**1**	**2**	**8 h**	**0**	**$BF_3 \cdot OEt_2$**	**Only β**	**<10**
5[b]	1	2	24 h	0	$BF_3 \cdot OEt_2$	82/18	
6[b]	1	2	8 h	25	$BF_3 \cdot OEt_2$	80/20	
7[c]	1	2	8 h	0	TMSOTf	80/20	
8[c]	1	2	8 h	0	NIS/TfOH	75/25	

Note: Bold values represents the optomized conditions.
[a]Anomeric composition of reaction mixture was monitored by ^{1}H NMR (300 MHz, CDCl$_3$), based on the integration of peaks.
[b]Longer time reduces the selectivity, increases temperature gives extra side products and less yield.
[c]The selectivity is reduced, in addition to side products.
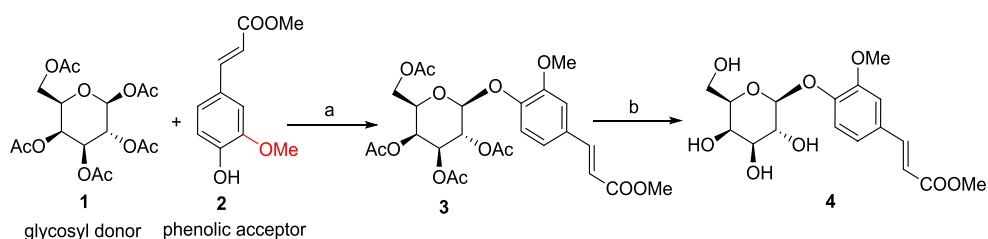
Scheme 1. Glycosylation reaction between **1** and **2**. Reagents and conditions: (a) BF$_3$·OEt$_2$, CH$_2$Cl$_2$, NEt$_3$ MS 4Å, 0 °C, 8 h, 70% yield; (b) NaOMe/MeOH, rt, 0.5 h, followed by Dowex (H$^+$), 88% yield.
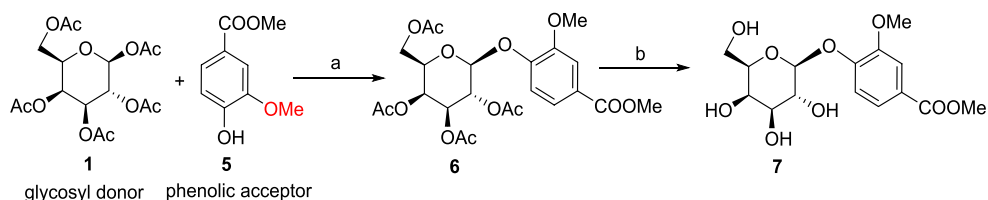
Scheme 2. Glycosylation reaction between **1** and **5**. Reagents and conditions: (a) BF$_3$·OEt$_2$, CH$_2$Cl$_2$, NEt$_3$ MS 4Å, 0 °C, 8 h, 80% yield; (b) NaOMe/MeOH, rt, 0.5 h, followed by Dowex (H$^+$), 90% yield.

vinyl protons of the C_α and C_β appeared at 6.30 and 7.60 ppm respectively. Additionally the value of coupling constant ($J = 15.0$ Hz) pointed to a *trans* position of the vinyl protons (*cis: J* = 12.0 Hz). Deacetylation of the corresponding **3** was carried out under mild catalytic methoxide/methanol (1 M), followed by neutralisation using dowex acidic (H$^+$) (Scheme 1). This step was considered very critical, due to labile property of the methyl ester under basic conditions. The target compound **4** was recrystallised using mixture of methanol and ethyl acetate solvents. The structure of **4** was confirmed on the basis of its HRFABMS, ^{1}H and ^{13}C NMR spectra. Complete proton and carbon chemical shift data for the products are given in (S1 see supplementary data).

The glycosylation of the methyl vanillate **5** proceeded smoothly under similar conditions using the same procedure (Scheme 2). The intermediate **6** was used directly. The formation of only β anomer of compound **7** was deduced from the NMR spectroscopy. Table 1 shows the values of the chemical shifts for ^{1}H NMR and ^{13}C NMR of the titles compounds.

2.2. Cytotoxic activity

Phenolic acids have been recognised not only for their properties as antioxidant compounds, but also for their cytotoxic effects towards breast cancer in *in vitro* and *in vivo* (Oliveira et al. 2011) and prostate cancer (Weng and Yen 2012; Seçme et al. 2015). The present work, aimed to study the cytotoxicity of ferulic acid methyl ester (FE), β-galactopyranosyl ferulic acid methyl ester (Gal-FE), vanillic acid methyl ester (VE) and β-galactopyranosyl vanillic acid methyl ester (Gal-VE) towards human breast cancer and prostate cancer cells. The objective was to identify whether the molecular alterations by the glycosylation of the test compounds would display more effective cytotoxic activity.

In the presents study, the anticancer activity of the compounds **2** (FE), **4** (Gal-FE), **5** (VE) and **7** (Gal-VE) was tested against two cell lines (MCF-7 and PC-3) and all of them showed

Table 2. Anticancer activity (IC$_{50}$, µM)[a] of compounds **2**, **4, 5** and **7** against the cell lines MCF-7 and PC-3.

Compound	IC$_{50}$ values (µM)	
	MCF-7	PC-3
(**2**)	896.9	324.2
(**4**)	373.7	359.9
(**5**)	529.3	344.3
(**7**)	236.7	729.1
Doxorubicin[b]	0.49	2.53

[a]The values of the IC$_{50}$ (concentration of drug yielding a 50% cell viability decrease).
[b]The drug doxorubicin was used as positive control.

moderate to good anticancer effects (Figure S29, see the supplementary material). As expected, the glycosylated compounds **4** and **7** were found to show higher anticancer activity on MCF-7 cells compared to its free esters **2** and **5** (Table 2). Conversely, compounds **2** and **5** showed noticeable decrease on PC-3 cell viability (IC$_{50}$ = 324.2 and 344.3 µM respectively) (Table 2). Surprisingly, Compound **7** caused growth inhibition at low dose in MCF-7 cells only with IC$_{50}$ observed of 236.7 µM with no significant inhibitory effect exerted onto PC-3. On the other hand, compound **2** showed the most potent cytotoxicity effect with IC$_{50}$ observed of 324.2 µM on PC-3 cells. Finally, (**5** and **7**) were less effective than (**2** and **4**) whereas most of the effects from (**2** and **4**) were observed on MCF-7 cells.

3. Conclusion

In summary, our findings, in agreement with data reported by other investigators, clearly indicate that glycosylated compounds (Gal-FE and Gal-VE) displayed a preferable cytotoxic activity against MCF-7 cancer cells than the aglycone esters. Therefore, glycosylation proved to be a promising method in modification of phenolic compounds and could serve as a novel prodrug. It is necessary to conduct further studies with other cell lines and *in vivo* animal models to discover the molecular alteration effect of the glycosylated phenolic compounds on prostate and breast cancer. In addition, the scope of our research will be extended to cover other active phenolic acids and include other sugars moieties such as glucose and mannose.

Supplementary material

Supplementary material relating to this article is available online alongside with experimental part (NMR data and cytotoxicity graphs)

Acknowledgements

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Nader Al Bujuq http://orcid.org/0000-0002-3089-7037

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