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Synthesis and Peptidyl Group Migration in Isopropylidene Acetals Derived from the Glucose-substituted Imidazolidinone Related to Opioid Peptide Leucine-enkephalin

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The reaction of the glucose-substituted imidazolidinone **1**, structurally related to endogenous opioid pentapeptide leucine-enkephalin (Tyr-Gly-Gly-Phe-Leu), with acetone in the presence of acid catalyst gives 2,3-*O*-isopropylidene derivative **2** along with the isomeric monoacetal **3**. Evidence is presented by using RP HPLC and NMR spectroscopy that, under the conditions of ketal formation, the peptidyl group migrated from the primary (**2**) to the adjacent secondary hydroxy group of the sugar moiety to give ester **3**. The product distribution in the equilibrated reaction mixture (**2** : **3** ≈ 1.5 : 1) indicated that 5-*O*-peptidyl derivative **2** is slightly more stable than ester **3**. Peptidyl group migration in aqueous solution at pH = 5 shows slow **2** → **3** rearrangement and markedly faster **3** → **2** conversion.

Key words: opioid, peptide, enkephalin, imidazolidinone, acetal, glucose.

INTRODUCTION

In earlier papers[1–3] we found that intramolecular rearrangement of the monosaccharide ester in which D-glucose, D-mannose or D-galactose, is linked, through the C-6 hydroxy group, to the C-terminal carboxy group of the endogenous opioid pentapeptide leucine-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH), in

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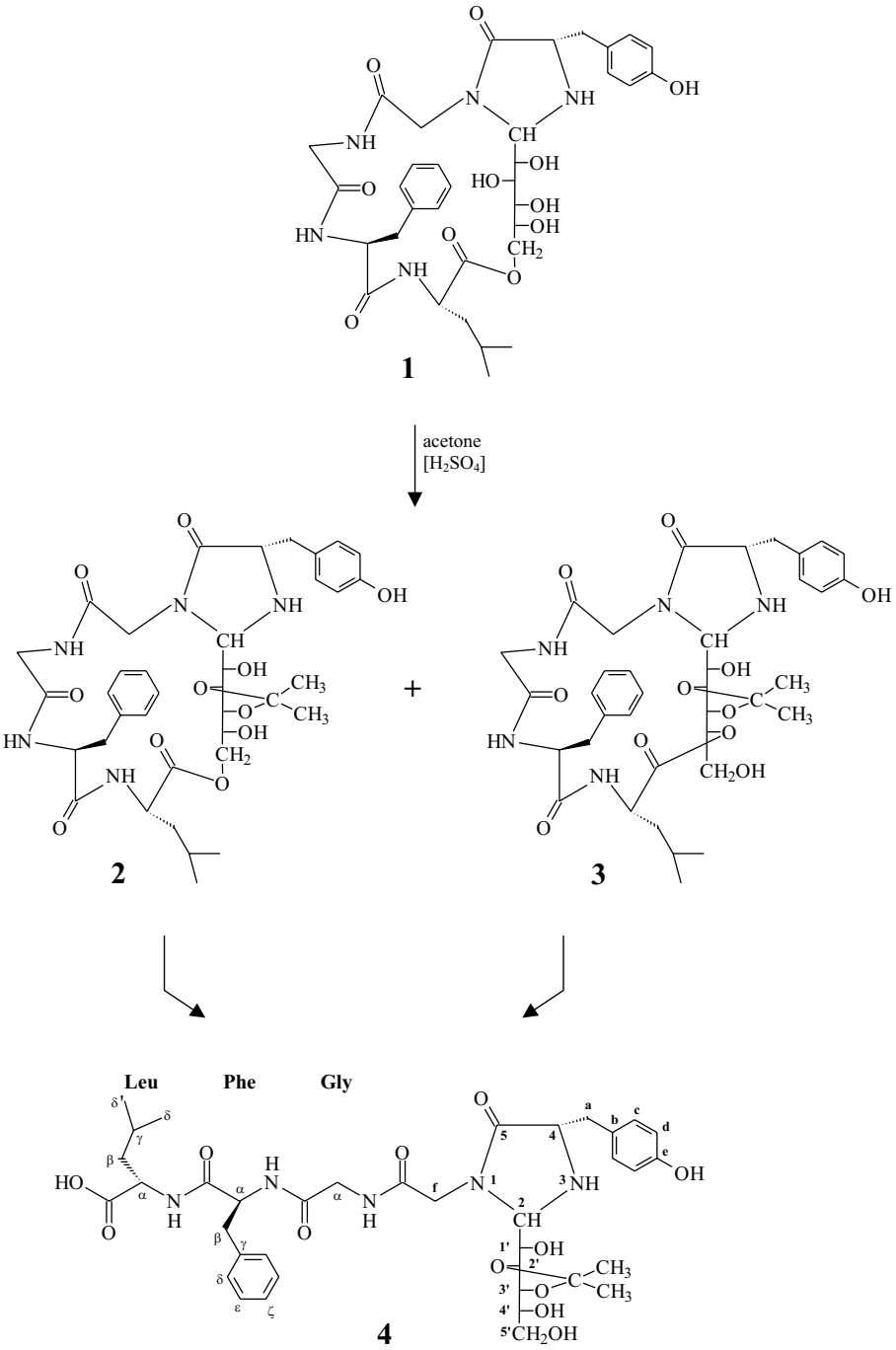
methanol as the solvent, resulted in the formation of an imidazolidinone in which C-1 of the glucose moiety forms a bridge between the α-amino group of the N-terminal tyrosine residue and the amide nitrogen of the Tyr-Gly peptide bond. Because of the new *N*,*N*'-acetal centre formed, the ring closure in D-glucose-related ester resulted in imidazolidinone diastereoisomers having *cis* and *trans* relative geometry of the substituents at the imidazolidinone ring moiety.[1,3] The imidazolidinone isomers were separated by RP HPLC. The major product, isolated in the yield of 35% and assumed to have *trans* configuration of the 2- and 4-substituents with respect to the plane defined by the heterocyclic moiety, is the bicyclic imidazolidinone **1**.

The structural features of compound **1** resemble those of peptide- and carbohydrate-containing macrocyclic compounds which have elicited much interest recently due to their potential to bind substrates in an enantioselective fashion and as efficient ion-transporters across model membranes.[4] To explore the reactivity of free hydroxy groups and utility for further synthetic transformations, herein we investigated the isopropylidenation of imidazolidinone **1**.

RESULTS AND DISCUSSION

Treatment of the imidazolidinone **1** in acetone with catalytic amounts of sulphuric acid under anhydrous conditions, for 4 h at room temperature, resulted in two new products which were separated by semipreparative RP HPLC to give isopropylidene derivatives **2** (20%) and **3** (24%) (Scheme 1). The structures of **2** and **3** were elucidated using ^{13}C NMR spectroscopy. The assignment of the signals (Table I) was made by comparison with those reported previously for the parent D-glucose-derived imidazolidinone **1**,[3] on the basis of literature data for isopropylidene acetals[5–8] and related acyclic sugar derivatives[9] as well as by using ^{1}H-^{13}C COSY experiments. The ^{13}C chemical shifts of the isopropylidene acetal carbon atom and of the methyl groups clearly indicated the presence of only one five-membered 1,3-dioxolane ring in **2** and **3** (Table I). In accordance with detailed studies by Buchanan *et al.*,[7,8] 1,3-dioxolane structures gave an acetal carbon signal at δ = 110–112 and two methyl signals at δ = 26–28 ppm whereas six-membered dioxane rings gave the acetal carbon at δ = 99–101 and two methyl groups at δ = 29–30 and 19–20 ppm. Formation of cyclic acetals leads to a rather large downfield shifts of the α-carbon atoms,[10] thus the 2',3'-position (see Scheme 1 for carbon atom numbering) of the isopropylidene group was deduced from chemical shifts of the respective carbon atoms in compounds **2** and **3**. The obtained results are in agreement with the observed preference for 1,3-dioxolane ring formation in alditols from the hydroxy groups having



Scheme 1.

TABLE I

^{13}C NMR data (DMSO-d_6) of imidazolidinone compounds **1–4**

Residue	Carbon atom[a]	δ / ppm			
		1[b]	**2**	**3**	**4**
Imidazolidinone	2	73.7	73.3	74.2	73.9
ring	4	59.3	60.6	59.3	59.3
p-hydroxybenzyl	a	34.3	35.7	34.6	34.7
	b	126.3	128.9	126.9	n.o.[c]
	c	130.5	130.1	130.6	130.4
	d	115.4	115.2	115.7	115.4
	e	156.6	156.1	156.8	156.6
N^1–CH_2	f	43.2	43.1	44.0	42.9
Sugar moiety	1'	67.2	69.1	65.2	66.9
	2'	71.7	78.7	80.4	79.2
	3'	72.3	76.5	74.3	76.4
	4'	69.4	70.0	76.3	73.3
	5'	66.8	64.1	60.7	63.3
Gly	α	42.2	42.2	42.7	41.8
Phe	α	54.9	54.4	53.9	53.8
	β	37.2	37.6	37.4	37.8
	γ	137.9	138.0	137.6	138.8
	δ	129.3	129.3	129.6	129.5
	ε	128.5	128.5	128.8	128.3
	ζ	126.7	126.5	127.2	126.6
Leu	α	50.8	51.7	52.4	50.4
	β	39.6	39.7	39.7	39.8
	γ	24.2	24.1	24.4	24.4
	δ	21.4	21.3	21.8	21.4
	δ'	23.1	22.9	22.9	22.9
Isopropylidene	CH_3		27.4	26.5	26.6
	CH_3'		27.4	27.6	27.2
	C		109.2	111.2	109.4

[a]See Scheme 1 for carbon atom numbering. [b]Data taken from Ref. 3. [c]n.o. = not observed.

threo configuration,[11] but interestingly, 1',2'-acetal of **1** was not detected in the isopropylidene products. The reason for this behaviour is not clear at present. In the ^{13}C NMR spectra of **3** downfield shift of the C-4' signal accompanied by a corresponding upfield shift of the C-5' signal was observed. This indicated that acyl (peptidyl) group migration from O-5' to O-4' had occured.

Hydrolysis of the ester bond in either **2** or **3** (0.1 M NH_4OH) gave a unique compound which was identified as 2',3'-*O*-isopropylidene derivative **4** (Scheme 1). The ^{13}C chemical shifts for **4** are summarized in Table I. Comparison of the C-4' and C-5' chemical shifts of **2** and **3** with that of **4** demonstrated an upfield shift of the respective carbon atom in the deprotected compound **4** owing to the loss of the deshielding effect of the acyl group.

Acyl migration to a neighbouring hydroxy group under acidic or basic conditions, *via* an *ortho* ester intermediate, has been well documented in the partially acylated (usually acetylated or benzoylated) carbohydrates.[12] Acyl groups generally rearrange from secondary to primary positions. To gain better insight into the migratory tendency of the bulky peptidyl group from the primary to the secondary hydroxy group, we next examined the relative amounts of the 5'-*O*- (**2**) and 4'-*O*-ester (**3**) present in the solution under the above conditions of the ketal formation. As evidenced by RP HPLC analysis after 1 h, the reaction mixture contained **2** and **3** in a 5 : 1 ratio whereas the product distribution obtained after 6 h, **2** : **3** ≈ 1.5 : 1, was found to represent essentially the equilibrium mixture and was not significantly changed up to 72 h. From the relative proportions of **2** and **3** detected in the acetalation mixture it is evident that at the beginning of the reaction the starting compound **1** is rapidly converted into 2',3'-*O*-isopropylidene derivative **2**. After 1 h, however, when **1** has been consumed, in the presence of the acid-catalyst, the amount of compound **3** started to increase indicating peptidyl residue migration from the primary to the adjacent secondary hydroxy group. The product distribution in the equilibrated mixture indicate that 5'-*O*-acyl derivative **2** is slightly more stable than **3**. To investigate the reversibility of the peptidyl group migration, the isomerization of the 5'-*O*-(**2**) and 4'-*O*-isomer (**3**) was followed in aqueous solution (pH = 5) at ambient temperature by using RP HPLC. Although isomerization of **2** is relatively rapid in acetone containing acid-catalyst, water is less efficient in promoting the migration. Thus, 5'-*O*-acyl derivative **2** is isomerized into **3** to the extent of only 14% after 8 days. However, the 4'-ester **3** is much more rapidly isomerized by water. The mixture obtained after 48 h contains 36% of **2** whereas the equilibrium mixture, attained in 8 days, contains 5'- and 4'-esters in the ratio ≈ 1 : 1, illustrating the migration from a secondary to a primary hydroxy group in a polar solvent environment.

EXPERIMENTAL

Melting points were determined on a Tottoli (Büchi) apparatus and are uncorrected. Optical rotations were measured at room temperature using an Optical Activity LTD automatic AA-10 Polarimeter in a 1 dm cell; concentrations are given in g/100 mL. RP HPLC was performed on a Varian 9010 HPLC system with a Eurospher 100 reversed-phase C-18 semipreparative column (250 × 8 mm I. D., 5 μm) under isocratic conditions by using different concentrations of MeOH in 0.1% trifluoroacetic acid (TFA), flow rate 1.1 mL. UV detection (Varian Model 9050 variable-wavelength UV-Vis detector) was performed at 280 nm. NMR spectra were recorded on a Varian Gemini spectrometer, operating at 300.1 MHz for ^{1}H and 75.5 MHz for ^{13}C nuclei. All samples were measured in DMSO-d_6 solution at 25° C in 5 mm NMR tubes. Chemical shifts, in ppm, are referred to TMS. Elemental analyses were carried out at the Microanalytical Laboratory, Ruđer Bošković Institute.

*cyclo-[*N-*({2-[-5)-D-*gluco-*Pentitol-1-yl]-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl-(1 → O}acetyl)glycyl-L-phenylalanyl-L-leucyl-] (**1**)*

Compound **1** was obtained under the conditions described by Horvat *et al*.[3] In brief, 6-*O*-(L-tyrosylglycylglycyl-L-phenylalanyl-L-leucyl)-D-glucopyranose[13] was incubated in dry MeOH for 3 days at 60 °C. The solvent was evaporated and the residue was purified by semipreparative RP HPLC using 47% MeOH/0.1% TFA as the eluent to give the pure imidazolidinone **1** (yield 35%, major isomer, retention time: 25.7 min) and the corresponding minor isomer (yield 10%, retention time: 24.4 min). Desalting on a Dowex 1X2, 200 (Ac) column (10 × 0.8 cm) and lyophilization gave pure **1** which was used in the subsequent experiments.

*cyclo-[*N-*({2-[-5)-2,3-*O-*Isopropylidene-D-*gluco-*pentitol-1-yl]-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl-(1 → O}acetyl)glycyl-L-phenylalanyl-L-leucyl-] (**2**)*
and
*cyclo-[*N-*({2-[-4)-2,3-*O-*Isopropylidene-D-*gluco-*pentitol-1-yl]-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl-(1 → O}acetyl)glycyl-L-phenylalanyl-L-leucyl-] (**3**)*

Bicyclic imidazolidinone **1** (40 mg, 0.06 mmol) was added at 0 °C to a solution of acetone (4 mL) containing H_2SO_4 (0.01 mL). The solution was stirred under anhydrous condition for 4 h at room temperature and the products precipitated by addition of cold ether. The precipitate was centrifuged off and purified by semipreparative RP HPLC using 52% MeOH/0.1% TFA as the eluent to give pure isopropylidene derivatives **2** (retention time: 24 min) and **3** (retention time: 22 min) which were crystallized from MeOH / diisopropyl ether.

Compound **2**: 10 mg (20%), m.p. 140–150 °C (decomp.), $[\alpha]_D = -34°$ ($c = 1$ in MeOH). For ^{13}C NMR data see Table I.

Anal. Calcd. for $C_{37}H_{49}N_5O_{11} \cdot CF_3COOH$ (M_r = 853.84): C 54.86, H 5.90, N 8.20%; found: C 54.99, H 5.92, N 8.04%.

Compound **3**: 12 mg (24%), m.p. 140–150 °C (decomp.), $[\alpha]_D = -50°$ ($c = 1$ in MeOH). For ^{13}C NMR data see Table I.

Anal. Calcd. for $C_{37}H_{49}N_5O_{11} \cdot CF_3COOH$ (M_r = 853.84): C 54.86, H 5.90, N 8.20%; found: C 55.00, H 5.98, N 8.34%.

N-*{[2-(2,3-*O*-Isopropylidene-D-*gluco*-pentitol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin–1-yl]acetyl}glycyl-L-phenylalanyl-L-leucine (**4**)*

Compound **2** (or **3**) (24 mg, 0.028 mmol) was dissolved in 0.1 M NH_4OH (5 mL) and stirred for 2 h at room temperature. The solvent was removed and the residue was purified by RP HPLC using 52% MeOH/0.1% TFA as the eluent to afford pure **4** (retention time: 15 min): 11 mg (52%), m.p. 125–135 °C (decomp.), $[\alpha]_D = -20°$ ($c = 1$ in MeOH). For ^{13}C NMR data see Table I.

Anal. Calcd. for $C_{37}H_{51}N_5O_{12}$ (M_r = 757.83): C 58.64, H 6.78, N 9.24%; found: C 58.70, H 6.99, N 9.38%.

*RP HPLC Analysis of the Peptidyl Group Migration in Compounds **2** and **3***

Isopropylidene derivatives **2** and **3** (1 mg) were each dissolved in water (1 mL) and kept at ambient temperature. The progress of the peptidyl group migration was monitored in aliquots removed every 24 h from the incubation mixtures, over a period of 8 days. The respective samples were directly analysed by RP HPLC by using 52% MeOH/0.1% TFA as the eluent.

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SAŽETAK

Priprava i premještanje peptidne skupine u izopropilidenskim acetalima dobivenima iz glukozom supstituiranog imidazolidinona, srodnoga opioidnom peptidu leucin-enkefalinu

Maja Roščić i Štefica Horvat

Reakcija glukozom supstituiranog imidazolidinona **1** (strukturno srodnoga endogenom opioidnom pentapeptidu leucin-enkefalinu, Tyr-Gly-Gly-Phe-Leu) s acetonom u prisutnosti kiselog katalizatora rezultira nastajanjem 2,3-*O*-izopropilidenskog derivata **2** i izomernog monoacetala **3**. Uporabom RP HPLC i NMR spektroskopije dokazano je, pod uvjetima priređivanja ketala, premještanje peptidne skupine s primarne (**2**) na susjednu sekundarnu hidroksilnu skupinu u šećernom dijelu molekule koje rezultira esterom **3**. Raspodjela reakcijskih produkata u ravnotežnoj smjesi (**2** : **3** ≈ 1,5 : 1) pokazuje da je 5-*O*-peptidil-derivat **2** nešto stabilniji od estera **3**. Ispitivanje premještanja peptidne skupine u vodenoj otopini pri pH = 5, pokazuje polaganu pregradnju **2** → **3** i znatno bržu pregradnju **3** → **2**.