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Synthesis and characterization of pregabalin lactose conjugate degradation products

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Abstract

Seven degradation products observed in formulated pregabalin have been characterized. These compounds result from Maillard reactions and Amadori rearrangements. Heating pregabalin in the presence of lactose formed significant quantities of these degradation products. The seven compounds corresponding to the observed degradation products were isolated by preparative liquid chromatography. The synthesis, isolation, and spectral characterization of the degradation products are detailed. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Malliard; Amadori rearrangement; Pharmaceutical analysis; HPLC; LC/MS/MS; Pregabalin

1. Introduction

In the long-term stability testing of formulated pregabalin (CI-1008) capsules, four major peaks corresponding to degradation products were observed in high-pressure liquid chromatography (HPLC) chromatograms. These products are described as degradation products formed by reaction of pregabalin with the excipient lactose (Fig. 1) in the pregabalin capsules. Fig. 2 shows an HPLC Chromatograph of Pregabalin 25 mg capsules stored 40 °C per 75% RH for 6 months with the four peaks, Unknowns A, B, C, and D, labeled. Further investigation using a more specialized HPLC method revealed that Peaks B and C arise from the coelution of multiple peaks. Peak B is the coelution of PD 0312236 and 0312237. Peak C is the coelution of PD 0224377, 0310886, and 0310887. Fig. 3 shows an HPLC chromatogram of pregabalin 25-mg capsules (6 months at 40 °C per 75% RH) using a specialized HPLC method with seven lactose conjugates labeled (PD 0224378, 0224377, 0312236, 0312237, 0310806, 0310886, and 0310887).

Primary and secondary amines are known to form conjugates with lactose by undergoing a Maillard reaction [1,2]. Scheme 1 shows the Maillard reaction of β-lactose with a primary amine. The product of this reaction is a simple glycosylamine, which is a combination of the lactose and the amine after a net loss of water. Maillard reaction products readily undergo an Amadori rearrangement to produce 1-amino-1-deoxy-2-ke-

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toses, which exist in solution as a mixture of pyranose and furanose forms in equilibrium (Scheme 2) [3,4]. The seven degradants identified in formulated pregabalin were determined to be conjugates of pregabalin resulting from Maillard reactions. Heating pregabalin in the presence of lactose formed significant quantities of these by-products. These compounds were isolated by preparative liquid chromatography and studied by mass spectrometry and NMR spectroscopy methods that led to the structural assignments shown below (Scheme 3). Four of these conjugates (PD 0224377, 0310806, 0310886, and 0310887) are monosaccarides, resulting from the Maillard reaction and Amadori rearrangement of pregabalin with either the galactose (PD 0224377 and 0310806) or the glucose (PD 0310886 and 0310887) moiety of lactose. The synthesis, isolation, and spectral characterization of the seven by-products are described in this report.

2. Experimental

2.1. Materials

Pregabalin was used as received from Pfizer Global Research and Development (Ann Arbor, MI). All other reagents and solvents were of analytical or HPLC grade. The solid chemicals were purchased from E. Merck (Darmstadt, Germany) and the solvents from Mallinckrodt (Paris, Kentucky).

2.2. Apparatus

The mass spectra were run on a Micromass Quattro II (Manchester, UK) triple quadrapole mass spectrometer using electrospray ionization (ESI). Nuclear magnetic resonance (NMR) experiments were performed at ambient temperature on a Varian INOVA 400 (Palo Alto, CA) operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR. Analytical scale chromatography was performed using a Perkin–Elmer (Norwalk, CT) Series 200 LC pump, Series 200 autosampler, and LC-235 diode array detector. Analytical separations were performed on a YMC ODS-AQ column, 100 × 2.1 mm, 5 μm particle size at 30 °C. Mobile phase consisted of 15 parts acetonitrile, ten parts methanol, and 75 parts 0.1% formic acid, which was delivered at a rate of 0.25 ml/min. Preparative scale chromatography was performed using a Varian Dynamax SD-1 solvent delivery system, SD-300 sample introduction pump, UV-1 detector, and an Isco (Lincoln, NE) Foxy 200 fraction collector. Mobile phase was delivered at 25 ml/min through a Dynamax C-18 250 × 41 mm, 8 μm column at ambient temperature.

2.3. Synthesis of crude pregabalin lactose conjugates

Pregabalin (0.8 g) and lactose (3.8 g) were dissolved in 5 ml of water with stirring and heat. The solution was then heated overnight at 90 °C in an open Pyrex™ beaker using a heating block. The resulting solid was then redissolved in approximately 20 ml of isopropyl alcohol by sonicating and heating. About 40 ml of acetonitrile were added to this solution. The resulting solid was subjected to preparative scale reversed-phase chromatography.

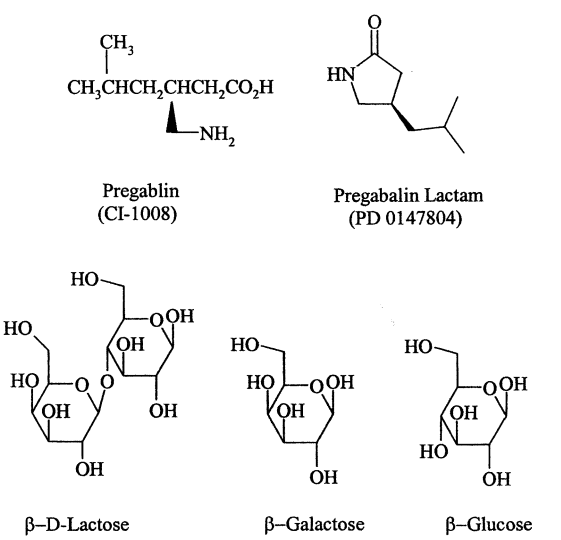


Fig. 1. Structures of pregabalin, pregabalin lactam, β-lactose, and β-lactose monomers (β-galactose and β-glucose).

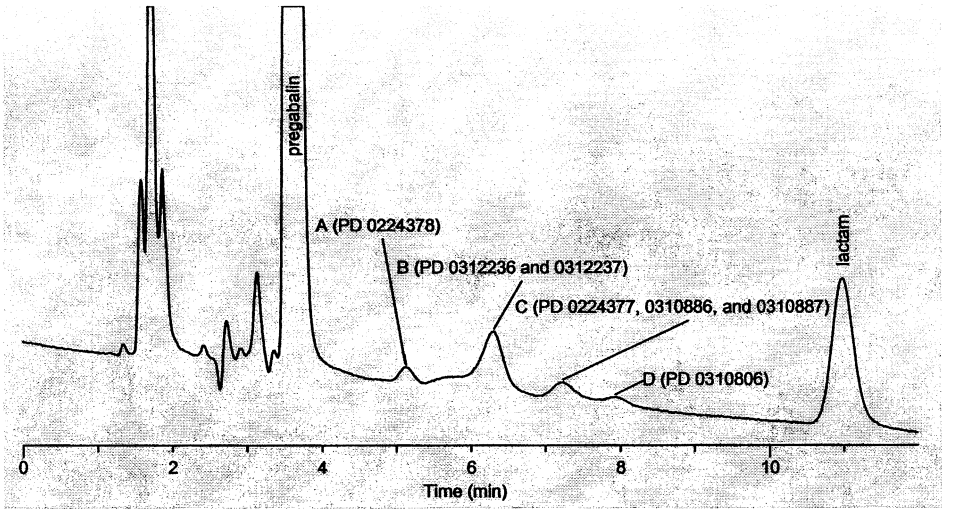


Fig. 2. HPLC chromatogram of pregabalin 25 mg capsule stored at 40 °C per 75% RH for 6 months original method HPLC conditions; column, waters μ-Bondapak 10 μ C18, 300 × 3.9 mm ID; mobile phase, 550:350:100:1 H_2O:MeOH:CH_2ON:pH 7.0 phosphate buffer; flow rate, 1.0 ml/min; detection, 210 nm; temperature, ambient.

2.4. Purification of pregabalin lactose conjugates

The solid material obtained above was subjected to reversed-phase preparative chromatography by injecting 20 ml of a saturated solution in mobile phase onto the preparative system described in Table 1. About 50 ml fractions were collected beginning at 150 ml of eluent. Fractions were analyzed by HPLC and those corresponding to the peaks of interest were pooled. The pooled fractions were concentrated on a rotoevaporator to remove the acetonitrile and methanol prior to lyophilization.

2.5. LC/MS experiments

Sample introduction and ionization was by ESI in the positive ion detection mode. Source cone voltage of 20 V, capillary voltage of 3.5 kV, and a source temperature of 90 °C with drying gas set to 450 l/h and nebulizing gas set to 35 l/h were the ionization parameters. The initial scan rate was 2.0 s per decade over a mass range of 50–1250 amu. Scan data was acquired using MASSLYNX multitasking operating system, version 3.2. The mass spectrometer was operated in MS/MS mode using argon as the collision gas at an indicated gas cell pressure of 1.5×10^{-3} torr and collision energy of 25 eV. Sample solutions were monitored in full scan, product, precursor, and neutral loss scanning modes.

2.6. NMR experiments

NMR experiments were performed at ambient temperature on a Varian INOVA 400 (Palo Alto, CA) operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR. All samples were run in D_2O. Typical sample size was 10–15 mg. All spectra were referenced to residual HDO at 4.63 ppm. Typically, ^{1}H, ^{13}C, APT (Attached Proton Test), gCOSY, gHMQC, gHMBC, and TOCSY NMR data were obtained for each conjugate.

3. Results and discussion

3.1. PD 0224378

Isolated PD 0224378, Lot P was 79% pure by HPLC area normalization. The relative response factor determined for PD 0224378 versus pregabalin in the capsule method is 15.9. Signals in the ^{1}H-NMR spectrum corresponding to the

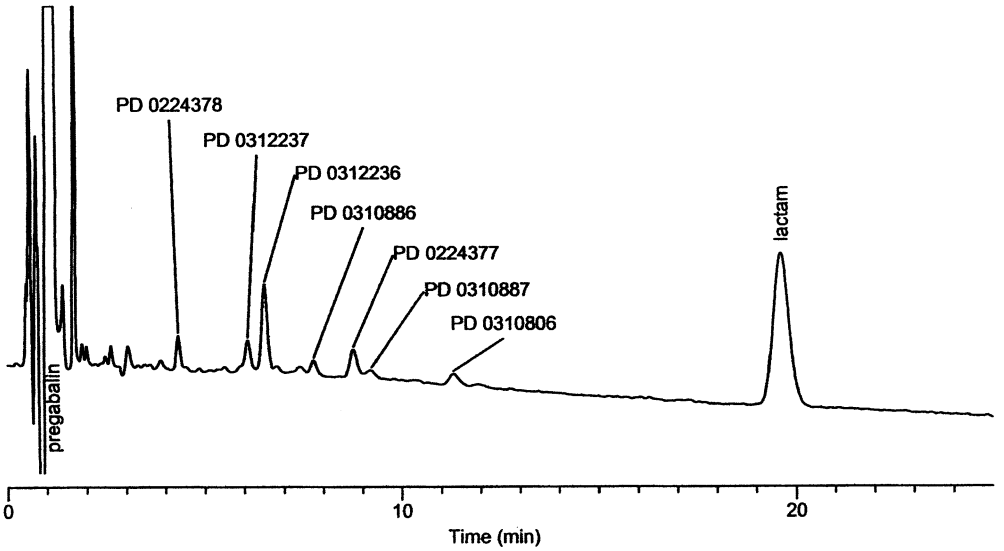
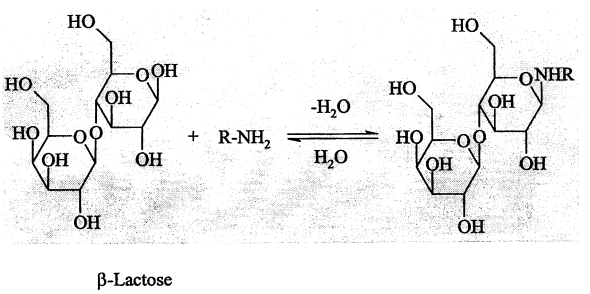


Fig. 3. HPLC chromatogram of pregabalin 25 mg capsules stored at 40 °C per 75% RH for 6 months modified method HPLC conditions; column, phenomenex luna C18 (2) 100 × 4.6 mm ID, 3 μm; mobile phase, 85:15 0.05%; formic acid, CH_3CN; flow rate, 1.5 ml/min; detection, 210 nm; temperature, ambient.

cyclic methylene (2.1, 2.4 ppm pair adjacent to the carbonyl; 3.0, 3.5 ppm pair adjacent to the nitrogen), cyclic methine (2.4 ppm), exocyclic methylene (1.2 ppm) and isopropyl (1.4 and 0.7 ppm) protons in the pregabalin moiety of the molecule are consistent with the closed-ring (lactam) form. The anomeric protons for the galactose (4.28 ppm) and glucose (4.87 ppm) moieties of lactose are also observed in the proton spectrum. In the ^{13}C-NMR spectrum, the cyclic methylene (39 and 50 ppm), cyclic methine (30 ppm), exocyclic methylene (44 ppm), isopropyl (27 and 22 ppm) and carbonyl (180 ppm) signals arising from pregabalin lactam are present. The anomeric carbons for the galactose (102.9 ppm) and glucose (80.3 ppm) moieties are also observed with their expected multiplicities. The profile of carbon signals in the glucose moiety (80.3, 69.5, 75.3, 77.6, 76.9, and 59.9 ppm for C1–C6, respectively) is consistent with the β-anomer [1]. The ion at m/z 466 in the mass spectrum (ESI +) of PD 0224378 arises from the protonated molecule. The mass of m/z 465 is consistent with the formula for PD 0224378, $C_{20}H_{35}NO_{11}$. The product ion spectrum is consistent with the proposed structure. An ion at m/z 142 corresponding to protonated pregabalin lactam is present. PD 0224378 is the lactam form of the glycosylamine formed by the Maillard reaction of pregabalin and lactose (β-anomer). Since the Maillard reaction only occurs with reducing carbohydrates and amines (primary or secondary) [1–3], it is assumed that lactam formation in pregabalin conjugates follows the initial Maillard reaction.

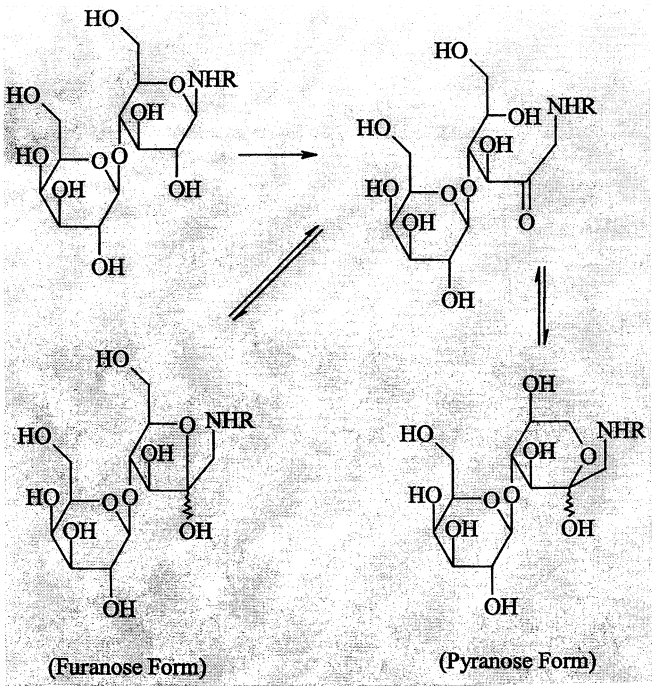
3.2. PD 0312236 and 0312237 in a 3:1 mixture

PD 0312236 and 0312237 were isolated in a 3:1 mixture. This mixture of isomers (furanose, pyra-



β-Lactose

Scheme 1. Maillard reaction of β-lactose with a primary amine.



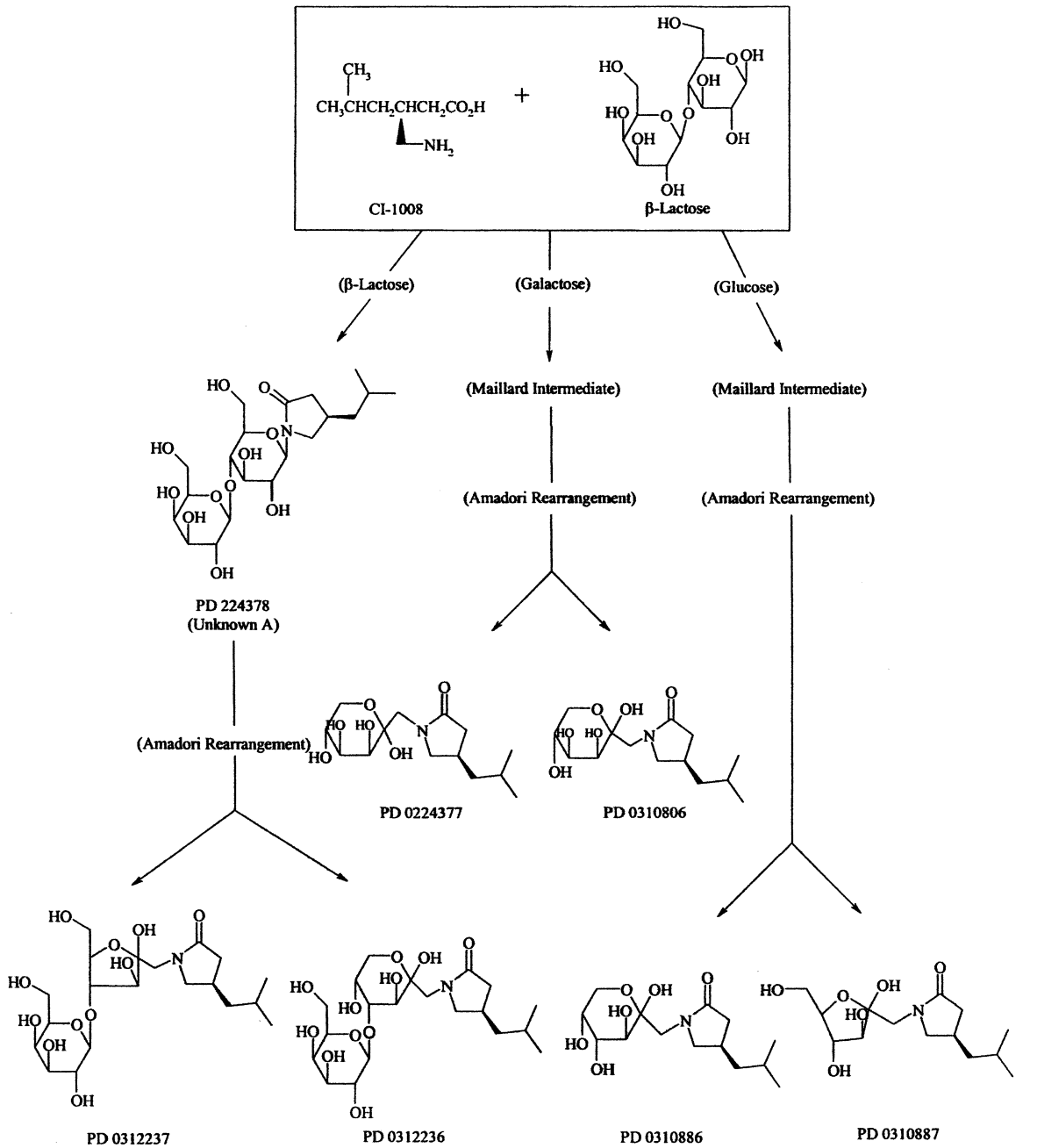
Scheme 2. Amadori rearrangement.

nose) was designated PD 0224379. PD 0224379, as a 3:1 mixture, is identical to the single peak referred to as unknown B in the method for pregabalin capsules. The relative response factor for PD 0224379 versus pregabalin is 20.2. The anomeric galactosyl protons for PD 0312236 and 0312237 are observed at 4.3 and 4.4 ppm in the proton spectrum. No other anomeric protons are observed. In the carbon spectrum, nonproton bearing carbons are observed at 98.6 and 102.4 ppm, in addition to the galactose anomeric carbons at 100.9 and 103.4 ppm (1 proton attached). The profile of carbon signals in the arabinosyl moiety of PD 0312237 (102.4, 76.5, 84.3, 80.0, and 62.7 ppm for C1–C5, respectively) is consistent with the β-furanose form. The profile for the arabinosyl moiety of PD 0312236 (98.6, 67.7, 77.3, 66.7, and 63.1 ppm for C1–C5, respectively) is consistent with the β-pyranose form [5]. The chemical shifts arising from the pregabalin moiety in the proton (0.7–3.6 ppm) and carbon (20–60 ppm, aliphatic) spectra are consistent with the lactam (closed-ring) form of the molecule. The ion at m/z 466 in the mass spectrum (ESI +) of PD 0224379 arises from the protonated molecule. The mass of m/z 465 is consistent with the formula for PD 0224379, $C_{20}H_{35}NO_{11}$. An ion at m/z 142 corresponding to protonated pregabalin lactam is present. The product ion spectrum is consistent with the proposed structure.

3.3. PD 0224377

Isolated PD 0224377, Lot P was 70% pure by HPLC area normalization. The relative response factor determined for PD 0224377 versus pregabalin in the method is 28.1. Both anomeric protons are missing from the proton spectrum, and a nonproton bearing carbon at 98.6 ppm is observed in the ^{13}C spectrum. In the carbon spectrum, the profile of signals in the lyxose moiety (98.6, 70.6, 70.7, 66.2, and 62.4 ppm for C1–C5, respectively) is consistent with the α-anomer (pyranose form) [5]. The chemical shifts arising from the pregabalin moiety in the proton (0.7–3.6 ppm) and carbon (20–60 ppm, aliphatic) spectra are consistent with the lactam (closed-ring) form

of the molecule. The ion at m/z 304 in the mass spectrum (ESI +) of PD 0224377 arises from the protonated molecule. The mass of m/z 303 is consistent with the formula for PD 0224377, $C_{14}H_{25}NO_6$. An ion at m/z 142 corresponding to protonated pregabalin lactam is present. The product ion spectrum is consistent with the proposed structure.



Scheme 3. Lactose conjugates of pregabalin.

Table 1
Preparative HPLC conditions

Operating parameter	Description
Column	Dynamax, C_{18}, 8 μm, 100 mm guard + 250 × 41 mm
Mobile phase	15:10:75 acetonitrile:methanol:0.1% formic acid (PD 224378, 0312236, and 0312237 isolation) 550:350:100:1 H_2O:MeOH:CH_3CN:pH 7 buffer (PD 310806 and 224377) 850:150:1 H_2O:CH_3CN:pH 7 buffer (PD 310886 and 310887)
Column temperature	Ambient
Detector wavelength	None used
Injection volume	20 ml
Flow rate	25 ml/min
Run time	60 min

3.4. PD 0310806

Isolated PD 0310806, lot P was 76% pure by HPLC area normalization. The relative response factor determined for PD 0310806 versus pregabalin in the method is 30.6. Both anomeric protons are missing from the proton spectrum, and a nonproton bearing carbon at 98.3 ppm is observed in the ^{13}C spectrum. In the carbon spectrum, the profile of signals in the lyxose moiety (98.3, 72.1, 73.6, 69.5, and 61.9 for C1–C5, respectively) is consistent with the β-anomer (pyranose form) [5]. The chemical shifts arising from the pregabalin moiety in the proton (0.7–3.6 ppm) and carbon (20–60 ppm, aliphatic) spectra are consistent with the lactam (closed-ring) form of the molecule. The ion at m/z 304 in the mass spectrum (ESI +) of PD 0310806 arises from the protonated molecule. The mass of m/z 303 is consistent with the formula for PD 0310806, $C_{14}H_{25}NO_6$. An ion at m/z 142 corresponding to protonated pregabalin lactam is present. The product ion spectrum is consistent with the proposed structure.

3.5. PD 0310886 and PD 0310887

We isolated PD 0310886 and 0310887 in a 3:1 mixture using a modified HPLC method (see Fig. 3 for conditions). In the method for pregabalin capsules, the two compounds coelute along with PD 0224377. The relative response factor for PD 0310886 and 0310887 (3:1 mixture) versus pregabalin in this method is 30.2. No anomeric protons are observed in the proton spectrum, and nonproton bearing carbons at 98.6 and 101.5 ppm are observed in the ^{13}C spectrum. The profile of carbon signals in the arabinosyl moiety of PD 0310886 (98.6, 69.1, 69.5, 69.0, and 63.5 ppm for C1–C5, respectively) is consistent with the β-pyranose form. The profile for the arabinosyl moiety of PD 0310887 (101.5, 77.0, 74.3, 80.5, and 62.4 ppm for C1–C5, respectively) is consistent with the β-furanose form [5]. The chemical shifts arising from the pregabalin moiety in the proton (0.7–3.6 ppm) and carbon (20–60 ppm, aliphatic) spectra are consistent with the lactam (closed-ring) form of the molecule. The ion at m/z 304 in the mass spectra (ESI +) of PD 0310886 and 0310887 arise from the protonated molecule. The mass of m/z 303 is consistent with the formula for both PD 0310886 and 0310887, $C_{14}H_{25}NO_6$. An ion at m/z 142 corresponding to protonated pregabalin lactam is present. The product ion spectrum is consistent with the proposed structures.

3.6. Additional experiments

In separate experiments, pregabalin (300 mg) was reacted in 5 ml H_2O (adjusted to pH 11 with KOH) at 80 °C with glucose (600 mg) and galactose (600 mg). In the glucose reaction, a 3:1 mixture of PD 0310886 and 0310887 resulted. In the galactose reaction, a 5:1 mixture of PD 0224377 and 0310806 resulted.

4. Conclusions

We have shown that drug in the presence of lactose undergoes a Maillard reaction over time to form conjugates with lactose in formulated product. Seven of these conjugates, which contain

the lactam form of the pregabalin moiety, were generated in milligram to gram quantities by heating pregabalin in the presence of lactose in solution. The compounds were then isolated by preparative liquid chromatography. The assigned structures of the isolated lactose–lactam conjugates were consistent with the NMR and mass spectrometry data. Of the seven compounds identified, one compound (PD 0224378) is the Maillard reaction product of pregabalin and lactose. The lactose used in the reaction was originally the α-lactose. However, upon dissolution in the above reaction conditions, the lactose rapidly equilibrates between the α and α forms. The conjugate PD 0224378 is the β-anomer. Two of the compounds (PD 0312236 and 0312237) result from the Amadori rearrangement of PD 0224378. One product is the β-furanose form (PD 0312237) and the other is the β-pyranose form (PD 0312236). The remaining four conjugates are monosaccharides, resulting from the Maillard reaction/Amadori rearrangement of pregabalin with either the galactose (PD 0224377, 0310806) or glucose (PD 0310886 and 0310887) moiety of lactose. These experimental results support the structural assignments of the monosaccharide conjugates. It is not known whether these monosaccharides are formed as a result of Maillard reactions with monomers of hydrolyzed lactose or via an alternative mechanism.

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