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Structural elucidation of novel degradation product of brotizolam

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ABSTRACT

When the drug product of brotizolam (**1**) is decomposed according to the interview form and patent, hydrolysate (**2**) is obtained, but its physicochemical data are still missing. To elucidate the structure based on a spectroscopic approach, the above-mentioned degradation product was isolated and applied to the structural analysis. As a result, the structure of decomposed product was found to be different from that of **2**. In this report, its isolation and structure elucidation by NMR and MS spectra are described.

Brotizolam (**1**),[1] 2-bromo-4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine, is a sleep-inducing benzodiazepine drug, and is licensed as Lendormin® by Boehringer Ingelheim GmbH.[2]

The hydrolysate (**2**) derived from **1** was reported as an impurity of the drug product on the Interview Form (IF),[3] and was produced at ca. 0.8% by storage of the tablet at room temperature for 3 years, and also according to the different patent, at 1.7–3.2% by the tablet degradation at 60 °C and 75% relative humidity for 15 days[4] (Fig. 1). However we were not able to find the physicochemical data of **2** in the IF or patent literature.

Therefore, we attempted to decompose the drug product according to the patent, to obtain **2**, and to elucidate the structure of the decomposed impurity based on a spectroscopic approach.

When the Lendormin® tablet had been degraded for ca. 2 weeks according to the above mentioned patent manner,[4] LC/MS spectrum showed a few peaks besides that of **1**. Among them, we focused on the LC/MS peak at *m*/*z* 410.9679 which showed the isotope pattern including one Br and one Cl elements same as **2**. Because its production was at 0.45% area[5] and the amount was

Figure 1. Chemical structures of brotizolam (**1**), the hydrolysate (**2**), and the novel degradation product (**3**).

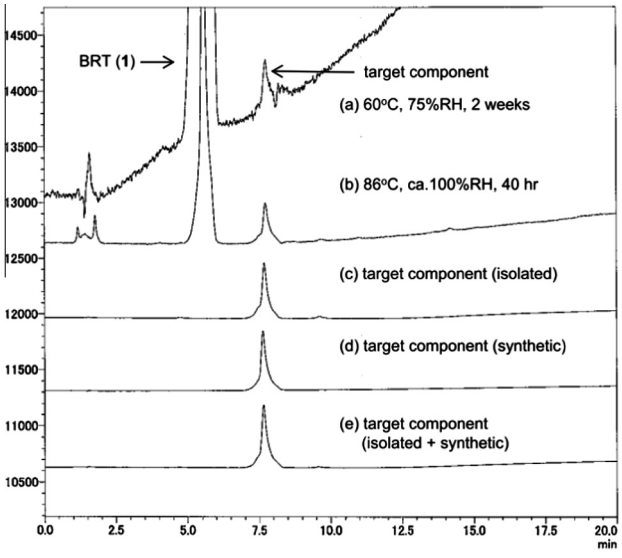

Figure 2. HPLC chromatogram of (a) the degraded drug product under 60 °C and 75% RH, (b) the degraded drug product under 86 °C and ca. 100% RH, (c) the target component isolated from the drug product degraded under 86 °C and 100% RH, (d) the target component synthesized from BRT and (e) mixture of the isolated and synthetic sample.

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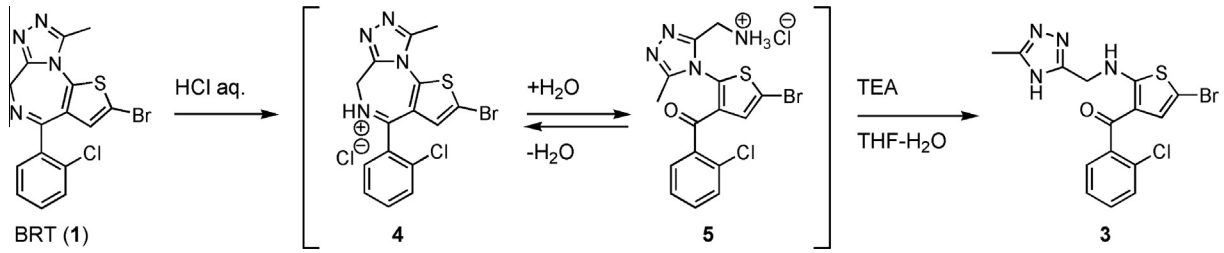

Scheme 1. Preparation of **3** from BRT (**1**) via an equilibrium mixture of the hydrochloride (**4**) and **5**.

Table 1
NMR assignments for **1**, **3** and benzoylthiophene (**6**)

No.	**1**		**3**		**6**	
	^1H[a]	^{13}C[a]	^1H[b]	^{13}C[b]	^1H[b]	^{13}C[b]
1						
2		108.6		93.3		93.1
3	6.89	128.5	6.51	129.5	6.45	129.0
3a		129.8		114.7		116.0
4		163.4		187.2		188.4
5			9.97		7.10	
6	4.87	46.3	4.54	45.1		
6a		152.4		158.2		
7						
8						
9		149.5		154.8		
10			13.4			
10a		136.0		168.6		166.4
1′		137.5		139.7		139.7
2′		131.3		130.5		130.5
3′		127.3	7.44	130.1	7.44	130.0
4′		131.2		130.6		130.6
5′	7.54 ~ 7.42 (4H)	129.6	7.41 ~ 7.30 (3H)	126.8	7.39 ~ 7.32 (3H)	126.7
6′		130.7		128.4		128.2
CH_3	2.62	11.3	2.28	11.9		

[a] Both ^1H and ^{13}C chemical shifts (ppm) in DMSO-d_6 were referenced to the tetramethylsilane (TMS) signal (0.00 ppm).
[b] Both ^1H and ^{13}C chemical shifts (ppm) in $CDCl_3$ were referenced to the tetramethylsilane (TMS) signal (0.00 ppm).

not sufficient to elucidate its chemical structure by NMR experiment, we explored conditions for better yield (Fig. 2).

When the degradation was carried out under the condition of 86 °C and ca. 100% RH for 40 h, the yield of the target component significantly-increased (at ca. 8.6% area). Accordingly, 500 tablets of Lendormin®, containing 125 mg of **1**, were degraded under the same condition, extracted with ethyl acetate, and purified by silica-column chromatography to give ca. 8 mg of the target component (yield: ca. 6%; LC purity: ca. 95% area).[6]

Concurrently, we examined the synthesis of the target component from BRT (**1**). Gallo et al. reported that the hydrolysis of **1** under aqueous HCl solution gave an equilibrium mixture of the hydrochlorides (**4**) and **5** inseparable from each other.[7] Surprisingly, when triethylamine (TEA) as a base affected the equilibrium mixture to deprotonate the ammonium of **5**, the target component was obtained (yield: 52%) in a quantity sufficient for NMR measurements[8] (Scheme 1).

The 1D and 2D NMR,[9] and MS/MS spectra of the synthetic sample were confirmed to be identical to those of the isolated sample from the degraded Lendormin® tablet. Therefore, we conducted the structural elucidation of the target component using the synthetic sample obtained from BRT (**1**).

First, in ^1H NMR experiment in $CDCl_3$, a total of 12 proton signals were observed at 13.4 (br s, 1H), 9.97 (t, 1H), 7.44 (dd, 1H), 7.41–7.30 (m, 3H), 6.51 (s, 1H), 4.54 (d, 2H) and 2.28 ppm (s, 3H). Among those, the two protons at 13.4 and 9.97 ppm proved to be exchangeable via hydrogen–deuterium exchange experiment with D_2O. Meanwhile, in ^{13}C NMR experiment, a total of 15 carbon signals were observed as shown in Table 1.

The COSY spectrum indicated that the proton at 9.97 ppm (t, 1H) coupled with the methylene proton at 4.54 ppm (d, 2H), which suggested that they were in the vicinal position. The protons at 7.44 (dd, 1H) and 7.41–7.30 (m, 3H) were assigned to the same aromatic ring protons. The singlet proton at 6.51 ppm remained to be determined.

The HSQC spectrum indicated that all the carbon signals observed at the following chemical shift values were classified as

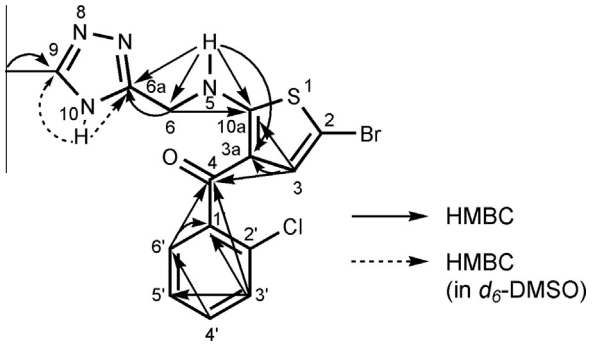

Figure 3. The HMBC of **3**.