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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**).

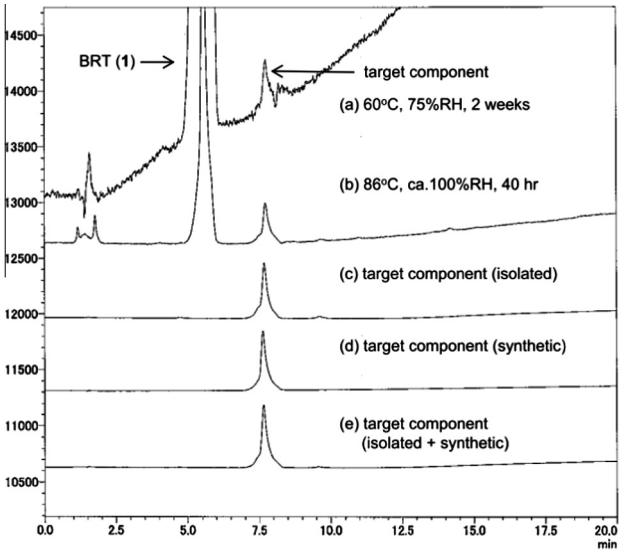

Figure 2. HPLC chromatogram of (a) the degraded drug product under 60 °C and 75% RH, (b) the degraded drug product under 86 °C and ca. 100% RH, (c) the target component isolated from the drug product degraded under 86 °C and 100% RH, (d) the target component synthesized from BRT and (e) mixture of the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

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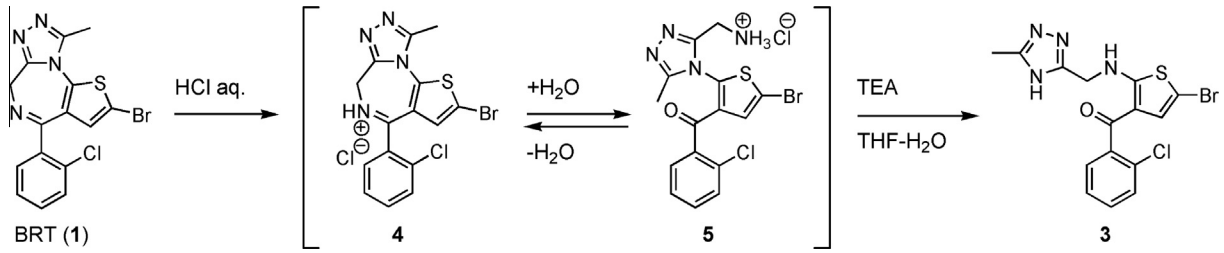

Scheme 1. Preparation of **3** from BRT (**1**) via an equilibrium mixture of the hydrochloride (**4**) and **5**.

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

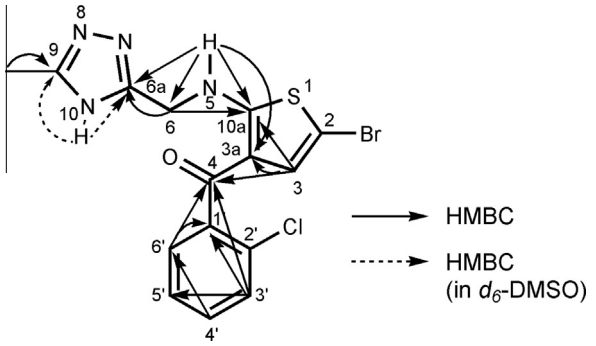

Figure 3. The HMBC of **3**.

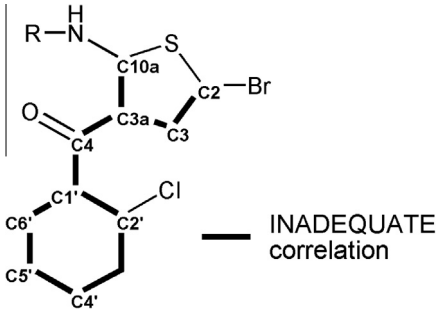

Figure 4. INADEQUATE correlation of **3**.

the quaternary carbons: 187.2, 168.6, 158.2, 154.8, 139.7, 130.5, 114.7 and 93.3 ppm. Moreover, the singlet proton at 6.51 ppm was assigned to the carbon at 129.5 ppm, and the methyl and methylene protons were assigned to the carbons at 11.9 and 45.1 ppm, respectively.

The cross peaks were observed in HMBC experiment as shown in Figure 3. The H-10 in $CDCl_3$ had a broadened peak on HMBC spectrum, while, in DMSO-d_6, the cross peaks were observed between H-10 and C-9, and C-6a. A series of the connectivity from the methyl proton to H-6 was also confirmed.

However, because there was no HMBC related to C-2 carbon at 93.3 ppm, the INADEQUATE experiment was conducted. The correlation shown in Figure 4 was observed, and C-2 carbon was found to be adjacent to C-3 carbon (see Fig. 5).

Finally, the chemical shift value 93.3 ppm of C-2 carbon was comparatively shifted to upfield from the corresponding signal of BRT at 108.6 ppm. To confirm the validity of this upfield shift, the benzoylthiophene (**6**) was prepared[10] and its NMR spectra were compared with those of **3** (Fig. 6). As a result, 1D NMR spectra and 2D NMR correlations of **6** were considerably consistent with those of the corresponding part of **3**, which suggests that the upfield chemical shift value of C-2 carbon is valid (Table 1).

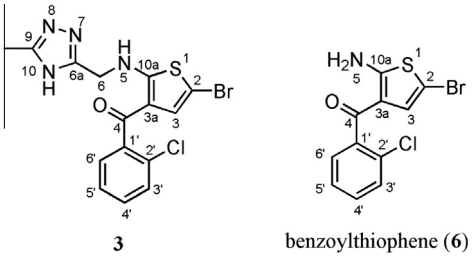

Figure 6. The location numbers in **3** and benzoylthiophene (**6**).

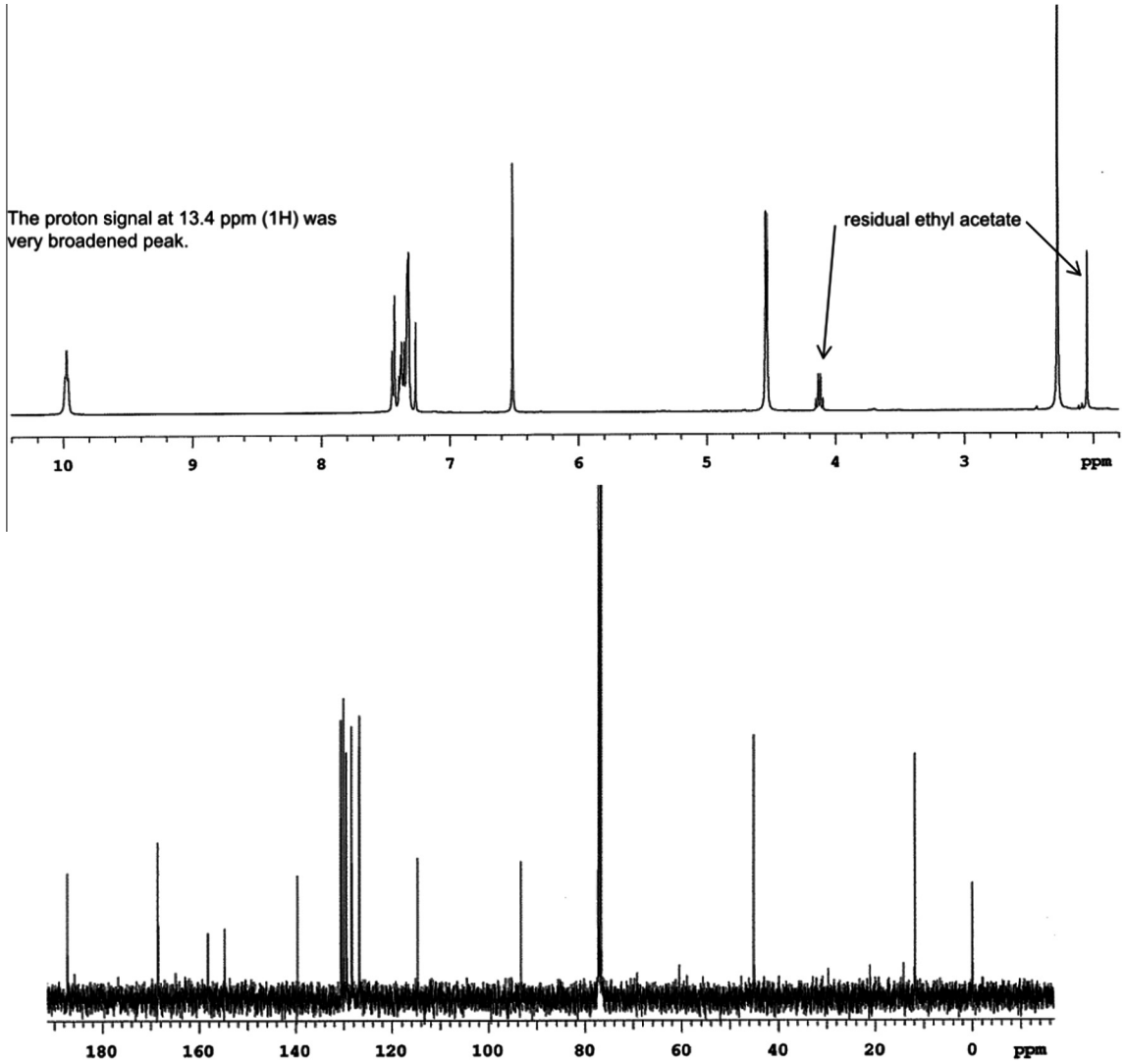

Figure 5. ^{1}H and ^{13}C NMR spectra of compound (**3**) prepared from BRT (**1**) (in $CDCl_3$).

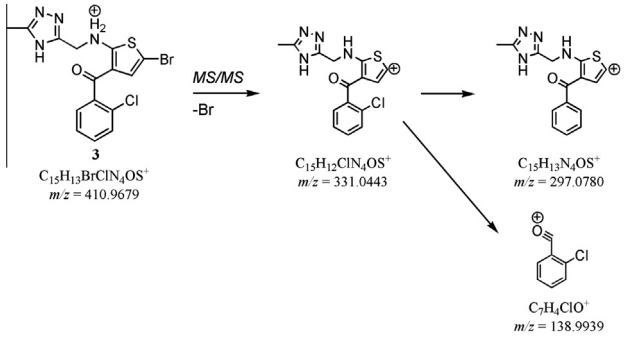

Figure 7. MS/MS fragment pattern of **3**.

The LC/MS spectra of **3** were indicated at *m*/*z* 410.9679, $[M+H]^+$ and *m*/*z* 408.9541, $[M-H]^-$ respectively, and the positive ion MS/MS spectrum of molecular related ion at *m*/*z* 410.9679 showed these product ions at *m*/*z* 331.0443, 297.0780 and 138.9939, respectively. The proposed fragmentation mechanism of these product ions is given in Figure 7.

In conclusion, we succeeded in the isolation, synthesis, and structural elucidation of the novel degradation product (**3**) that had been known as one of the impurity in the degraded BRT. The chemical structure of **3** was identified as 2-(5-methyl-4*H*-1,2,4-triazol-3-yl)methylamino-3-(2-chlorobenzoyl)-5-bromothiophene, which is different from the structure of hydrolysate (**2**).

Acknowledgment

We thank Professor Y. Ohfune (Osaka City University) for the valuable discussions about the degradation mechanism.

References and notes

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4. Yamaguchi, T.; Shimada, T.; Kume, H.; Kitada, A.; Utigaki, M.; Okuda, N. JP Patent 2,011,063,567, 2011.
5. *LC–mass spectrometry*: LC–MS system used was a Shimadzu LCMS–IT-TOF equipped with Prominence UFLC$_{XR}$ (Shimadzu, Japan). Waters SunFire™ C18 (4.6 × 150 mm, 5 μm) column was used with the following gradient condition of *T* (min)/%*B* (v/v): 0/40, 25/95, 35/95. The gradient involves two mobile phases consisting of $AcONH_4$ (10 mM) as solvent A and CH_3CN as solvent B. The LC–MS spectrum of the degradation product was performed with positive electro spray ionization (ESI^+) setting interface voltage at 4.5 kV and the capillary temperature at 200 °C. The collision gas for MS/MS experiment was used Ar gas.
6. *Isolation of **3** from the degraded drug product*: 500 Tablets of Lendormin® 0.25-mg made in Boehringer Ingelheim were grinded and powdered with a mortar, and then set in the container and kept at 86 °C and ca. 100% RH for sum 40 h (on this operation, you must pay attention to the sample, and, in some cases, stir it with a spatula, lest it becomes a dark-brown hard lump). The resultant milky-white powder was suspended in and extracted with ethyl acetate (600 mL) twice. After filtered to remove insoluble matters, the ethyl acetate layer was concentrated and purified by silica column chromatography (eluent: hexane/ethyl acetate) to give 8 mg of **3**.
7. Gallo, B.; Alonso, R. M.; Lete, E.; Badía, M. D.; Patriarche, G. J.; Gelbcke, M. *J. Heterocycl. Chem.* **1988**, *25*, 867.
8. *Preparation of **3** via HCl-hydrolyzed solution of BRT*: To BRT (**1**) (6.00 g, 15.2 mmol) in THF (60 mL), 1.3 N HCl aq (60 mL, 76.0 mmol) was added, and then stirred at 60 °C for ca. 5 h. After cooled to rt, TEA (7.69 g, 76.0 mmol) was added to the resultant solution and then stirred at rt overnight. The reaction solution was concentrated under reduced pressure and extracted with ethyl acetate. The organic layer was washed with water and satd. NaCl soln, dried over Na_2SO_4, concentrated under reduced pressure and purified by silica column chromatography (eluent: hexane/ethyl acetate) to give 3.27 g (52%) of **3** as greenish yellow amorphous. The ^1H and ^{13}C NMR spectra in $CDCl_3$ were shown in Figure 5.
9. *NMR spectrometry*: NMR measurements were performed at rt on Varian 400 MHz NMR spectrometer using $CDCl_3$ and DMSO-d_6 as solvent. Sample concentration was 1 mg in 0.6 mL for ^1H and ^{13}C, and 300 mg in 0.7 mL for inadequate experiment. The chemical shifts were referenced to TMS. All pulse sequences were applied by using the standard spectrometer software package.
10. *Preparation of **4***: To (*E*)-7-bromo-5-(2-chlorophenyl)-1*H*-thieno[2,3-*e*][1,4]diazepin-2(3*H*)-one (14.2 g, 40.0 mmol) in THF (142 mL) and H_2O (14.4 mL), *p*-toluenesulfonic acid-mono hydrate (6.89 g, 40.0 mmol) was added at rt, and then stirred at 65 °C for 30 h. After cooled to 0 °C, the reaction solution was quenched with TEA (8.10 g, 80.0 mol) and allowed to rt. After that, the quenched solution was concentrated and purified by silica column chromatography (eluent: hexane/ethyl acetate) to give 5.77 g (46%) of benzoylthiophene **4** as brown solid.