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Color change in Perlodel® tablets induced by LED lighting – photolysis of bromocriptine mesylate

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Various types of fluorescent lights are found in the dispensing rooms of medical facilities, such as hospitals and pharmacies, in Japan. However, to reduce electric power consumption, it was necessary to evaluate the substitution of fluorescent lighting with light emitting diode (LED) lighting, which has become widespread in recent years. We subjectively evaluated several types of medicines stored under various light sources and found that different color changes were induced in tablets. In this study, we focused on Perlodel® tablets, containing 2.5 mg bromocriptine mesylate, as an example for the objective evaluation of the differences in the color change of tablets when stored under LED lighting and fluorescent lighting. High-performance liquid chromatography (HPLC) analysis of part of the tablet surface area revealed a change from white to light brown or dark brown after 28 days of irradiation, with a residual concentration of bromocriptine mesylate of 85.5 % under fluorescent lighting, 85.6 % under daylight-color LED lighting, 90.3 % under bulb-color LED lighting, and 99.2 % in the dark. In addition, the ultraviolet (UV)-visible spectral study of the absorbance of a photo-product at 400–550 nm indicated that the color change of the Perlodel® 2.5 mg tablet was caused by photochemical degradation of bromocriptine mesylate. Thus, this analysis of the photochemical changes in drugs stored under different light sources demonstrated the potency of LED lights. Through the objective evaluation of the color change, the cause of the color change was determined; this will allow us to develop a strategy that minimizes possible disadvantages to patients, such as a decrease in treatment efficacy owing to decomposition of the main component or adverse caused by decomposed matter.

1. Introduction

The Japanese structural equipment standards for pharmacy dispensing rooms (Regulations for Buildings and Facilities for Pharmacies (Ministry of Health, Labor and Welfare, 1961)) indicate that there should be a "minimum of brightness of 60 lux for displaying ordinary medicines or on a dispensing table, and a minimum of 120 lux for a display table". In many dispensing rooms, fluorescent lighting fixtures that meet this structural equipment standard are used. However, in recent years, several types of LED lighting have become commercially available as lighting fixtures and their use has increased. In many countries, including the United States and Japan, incandescent lumps are being phased out; although the penetration rate is not high, the uptake of LED lighting has followed an increasing trend (U.S. Department of Energy, 2017). In the dispensing room, where it is expected that the lighting fixtures are switched on for many hours per day, the need for energy efficiency has demanded the consideration of the change to LED lighting suitable for prolonged usage.

We have evaluated the color change in several kinds of medicines after the light irradiation under LED and fluorescent lighting and reported that, even under LED lighting, there were a few medicines that experienced a color change (Yamashita et al. 2015, 2016 and 2018). Among them, Perlodel® 2.5 mg tablets of bromocriptine mesylate (Perlodel® 2.5 mg Tab.) were found to exhibit a remarkable color change (Yamashita et al. 2016, 2018). Perlodel® 2.5 mg Tab., a formulation of the ergot alkaloid derivative bromocriptine mesylate, exerts sustained dopaminergic action, acts on the hypothalamus/pituitary system to enable the endocrine system to inhibit secretion of prolactin and growth hormone, and acts on the nigrostriatal body to affect the central nervous system and cause anti-parkinsonian action. According to the data provided by Sun Pharmaceutical Industries Ltd., Perlodel® 2.5 mg Tab. is unstable when exposed to light and heat, but is stable at room temperature, and shows appearance changes when heated to 50 °C. In previous reports, it has been demonstrated that hydration of bromocriptine mesylate occurs following photoexcitation on the 9th and 10th positions when irradiated in solution and decomposition of bromocriptine mesylate occurs when irradiated with light in the solid state (Giron-Forest et al. 1979; Phakinee and Jankana 2013). Moreover, the presence of analogous substances, such as hydrolysis products, dehydrated compounds, and stereoisomerism, and metabolites has been observed and some are sold as reagents. However, there have been no reports on the structural determination of the decomposition products associated with light irradiation.

We also previously found that drugs stored under LED lighting tend to have fewer changes in color tone than fluorescent lamps (Yamashita et al. 2015, 2016 and 2018). In studies of three types of LED lighting, the following trend was observed in the degree of color change: bulb-color LED lighting < daytime-white LED lighting < daylight color LED lighting (Yamashita et al. 2016, 2018). However, the evaluation methods used so far have only been subjective evaluation by evaluators and evaluation by image analysis software and colorimeter (Yamashita et al. 2015, 2018). In previous reports, we examined Lasix® 20 mg Tab., Perlodel® 2.5 mg Tab., Nipolazin® 3 mg Tab., and Fluitran® 2 mg Tab. Among them, Perlodel® 2.5 mg Tab. exhibited the most obvious change in color. Moreover, to the best of our knowledge, there have been reports on the quantification of degradation products of montelukast, nifedipine and clavulanic acid under LED lighting, but studies with bromocriptine have not been conducted (Tania et al. 2015). Therefore, in this study, we performed an objective evaluation, which focused photostability of Perlodel® 2.5 mg Tab. after storage in LED and fluorescent lighting.

2. Investigations, results and discussion

2.1. Photostability of Perlodel® 2.5 mg Tab.

2.1.1. Light irradiation test

Perlodel® 2.5 mg Tab. taken from a press-through package (PTP) were placed under daylight-color LED lighting, bulb-color LED lighting, and fluorescent lighting at room temperature. Illuminance on the medicine was set to 1000 lux. Each medicine under each illumination was exposed for up to 28 days (approximately 670,000 lux·h). The control for comparison consisted of each medicine placed in the dark for the same number of days. When the authors observed the appearance of Perlodel® 2.5 mg Tab. after light irradiation, it was found that the degree of color changed occurred in the order of fluorescent lighting > daylight-color LED lighting > bulb-color LED lighting >> dark place. In addition, it was confirmed that the tendency observed in the previous report was reproduced. This study is a continuation of a previous report (Yamashita et al. 2016); however, in order to make the light irradiation conditions identical and the change in color tone more noticeable, the irradiation test was performed on medicines taken from PTP.

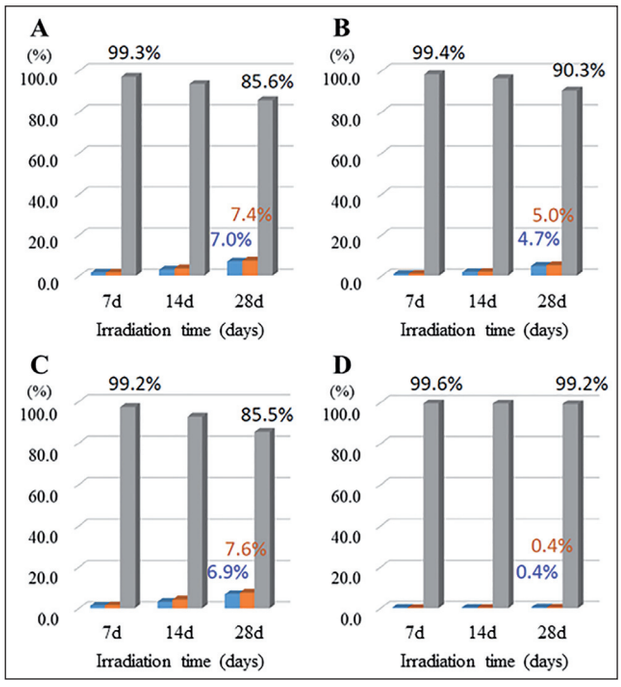

Fig. 1: Production rate of product after light irradiation
The residual ratios of the drug substance and the production rate of the product on the tablet surface of Perlodel® 2.5 mg Tab., which indicated the color change after the light irradiation test, are shown.
■RT=8.89 min (Product B), ■ RT=9.15 min (Product A), ■ RT=9.32 min (Bromocriptine Mesylate)

2.1.2. Evaluation of residual ratio of drug substance and production rate of product

The residual ratio of drug substance after scraping off the surface part with the color change and analysis by HPLC is shown in Fig. 1. Here, as bromocriptine has an absorption peak of approximately 305 nm (Japanese Pharmacopoeia), it is also absorbed when decomposed products, so the relative ratio to the absorbance at 254 nm can be used to evaluate the drug residual rate. The HPLC analysis revealed the drug residual rate after 28 days was 85.5 % under fluorescent lighting, 85.6 % under daylight-color LED lighting, 90.3 % under bulb-color LED lighting, and 99.2 % in the dark. In addition to the drug substance, two types of products were detected, with retention times (RTs) of 9.15 min and 8.89 min. The products with RT 9.15 min and 8.89 min were designated as products A and B, respectively. The production rate (relative ratio at 254 nm) was under fluorescent lighting (product A, 7.6 %; product B, 6.9 %), under daylight-color LED lighting (product A, 7.4 %; product B, 7.0 %), bulb-color LED lighting (product A, 5.0 %; product B, 4.7 %), and in the dark (product A, 0.4 %; product B, 0.4 %).
The production is dependent on the difference in light source because the vicinity of the base of the UV absorption band (approximately 305–325 nm; ε 7.3×10^3) of the drug substance depends on the degree of absorption of LED lighting. Therefore, it could be interpreted that lighting (fluorescent lighting and daylight-color LED lighting) with intense light emission in that part of the spectrum more readily resulted in a color change. In addition, the color change in appearance became larger under fluorescent lighting or daylight-color LED lighting, whereas the drug residual rate was lower under fluorescent light or daylight-color LED lighting. For this reason, it was considered that the color change was accompanied by decomposition of the drug substance.

2.2. Photostability of bromocriptine mesylate

2.2.1. Accelerated light irradiation test

To evaluate the photostability through the change in the amount of ingredients, as measured through various instrumental analyses, unnecessary and insufficient amounts were obtained for the samples from the surface of the Perlodel® 2.5 mg Tab. which had undergone color change as described in Section 2.1. We therefore investigated bromocriptine extracted from Perlodel® 2.5 mg Tab. Approximately 100 mg of bromocriptine bulk powder was dissolved in 100 mL of anhydrous acetonitrile and light-irradiated with a high pressure mercury lamp (Riko Rotary Photochemical Reactor, Model RH400-10W) through a bismuth chloride solution filter capable of blocking light of 300 nm or less without degassing the solution; after 7 h, the main component had almost disappeared.

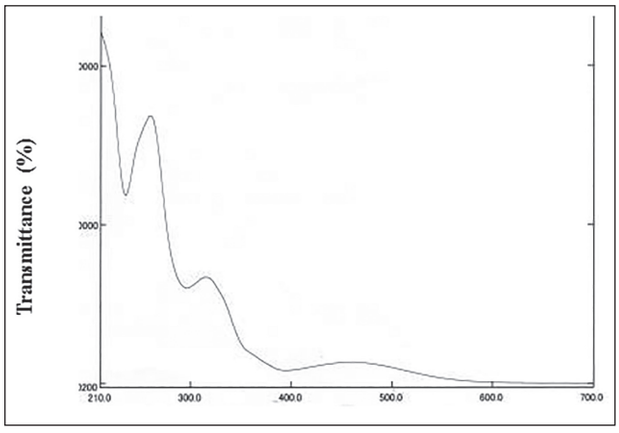

Fig. 2: UV-vis spectrum of product A (RT = 9.15 min) after irradiation
The results of measuring the product A (RT = 9.15 min) dissolved in methanol after irradiation with light with an UV- absorption spectrophotometer are shown. Absorption peaks are observed at approximately 259, 314, and 460 nm.

2.2.2. Product separation and evaluation

The reaction solution after the accelerated light irradiation test was concentrated under reduced pressure and the residue was separated on a silica gel column (eluent, chloroform/MeOH; gradient, 20:1 to 10:1). Fraction 4 (8.4 mg, mixture), fraction 5 (8.5 mg, almost pure), fractions 6–8 (11.3 mg, mixture), fractions 9–13 (5.6 mg, mixture), and fractions 24–27 (26.5 mg, pure product) were obtained. Of these, the product of fractions 24–27 was a reddish brown powder for which the UV-visible spectrum (Fig. 2) matched product A. Therefore, it was considered that product A (RT = 9.15 min) out of the products (RT = 8.99 and 9.15 min) extracted from the tablet surface changed in color to reddish brown, as described above. From the above, it was suggested that the cause of color change of Perlodel® 2.5 mg Tab. after light irradiation test was the decomposition of the main component.
In the accelerated light irradiation test, the drug almost disappeared after light irradiation for 7 h, compared with the case in which only

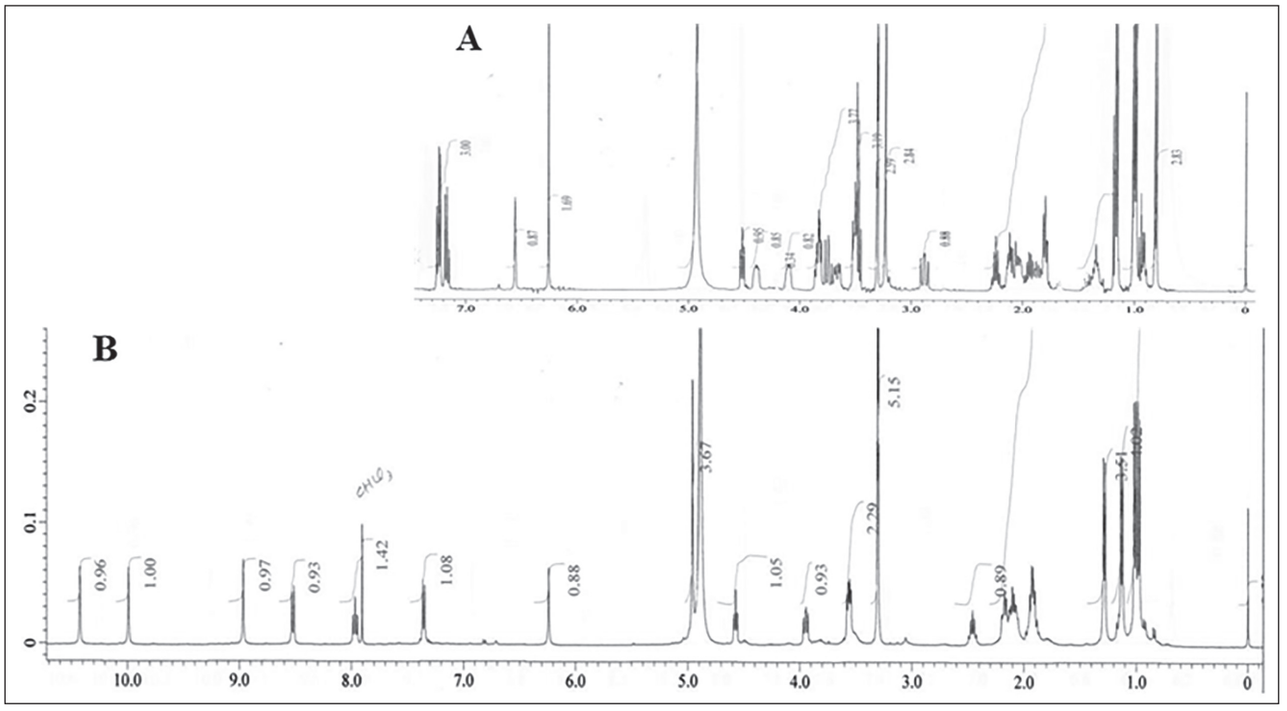

Fig. 3: ^{1}H-NMR spectrum of the bulk powder of bromocriptine and product A (RT = 9.15 min).
A: Bromocriptine bulk powder, B: product A (RT = 9.15 min).

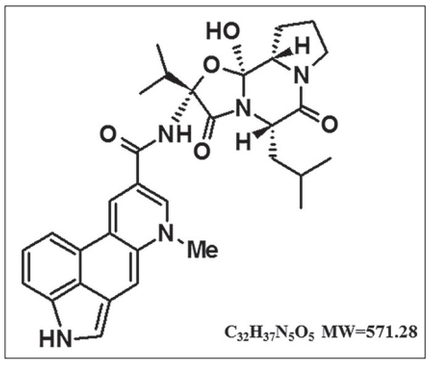

Fig. 4: Structural formula of photolysate of predicted bromocriptine bulk powder

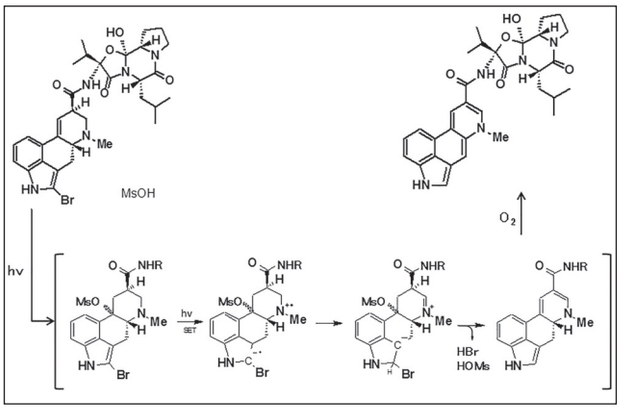

Fig. 5: Photolytic mechanism of predicted bromocriptine bulk powder

a small percentage of photodegradation occurred, even after continuous irradiation for 28 days. This indicated that the quantity of light absorbed by the UV absorption band of bromocriptine (approximately 305–325 nm; ε 7.3×10^3) greatly differed and that for a 400 W high pressure mercury lamp, the intensity of light at 313 nm and 365 nm was much greater than for LED lighting; therefore, after the UV absorption of bromocriptine (approximately 305–325 nm; ε 7.3×10^3), the photodecomposition reaction progressed gradually after low excitation of the lower wavelengths of the absorption band. In the light irradiation test, two types of products were detected, whereas in the accelerated light irradiation test, it was confirmed that several compounds were detected. It is presumed that secondary decomposition of photoproducts resulted from the high light intensity.

2.3. Estimation of structure and degradation mechanism of bromocriptine mesylate after photoirradiation

The nuclear magnetic resonance (NMR) spectrum of the product of fractions 24–27 obtained as described in 2.2.2 was measured and compared with that of bromocriptine; a large change was observed (Fig. 3). The NMR spectral analyses enabled the proposed the structure of the tetracyclic aromatic compound shown in Fig. 4. The mechanism of formation of this compound was thought to comprise the following steps (Fig. 5): the relative photostability of bromocriptine made the styrene moiety more susceptible to nucleophilic addition (Anderson et al. 1988); initially, methanesulfonic acid (MsOH) addition to the styrene moiety occurred under light irradiation conditions and formed a free N-Me group; single electron transfer from the group to the bromoindole ring was induced by light irradiation and resulted in the formation of a tetrahydrotetracyclic compound that was stable to light irradiation. However, as this tetrahydrotetracyclic compound has a distorted structure, it is thermally unstable. It is assumed that the tetracyclic aromatic rings were formed by the progressive removal of HBr and MsOH under the conditions used. It is understood that this final process includes auto-oxidation for further stabilization.

In order to support this chemical structure and decomposition mechanism, further investigation of the photoreaction mechanism and the chemical reactivity of the product is necessary. Therefore, our present results offer a general overview.

2.4. Conclusion

In this study, an objective evaluation of color change was demonstrated by using Perlodel® 2.5 mg Tab. as an example. The trends

observed by subjective evaluation were confirmed and suggested that the color change after light irradiation is a result of the photolysis of bromocriptine mesylate. We have examined only Perlodel® 2.5 mg Tab., but other bromocriptine formulations may change in color as well. Through the objective evaluation of the color change, the cause of the color change was determined; this will allow us to develop a strategy that minimizes possible disadvantages to patients, such as a decrease in treatment efficacy owing to decomposition of the main component or adverse events caused by decomposed matter.

3. Experimental

3.1. Pharmaceuticals

Perlodel® 2.5 mg Tab. (Sun Pharmaceutical Industries Ltd.) was used as the target drug.

3.2. Lighting fixtures

The lighting conditions were daylight-color LED bulb (Panasonic Corp., LDA 11 DG, Tokyo, Japan), bulb-color LED (Panasonic Corp., LDA 10 LG/Z 60 W, Tokyo, Japan), and bulb type fluorescent lighting (Panasonic Corp., EFA 15 EN 10 H 2, Tokyo, Japan). The color temperature of each light was 6700 K, 2700 K, and 5000 K, respectively.

3.3. Preparation of tablet sample

Approximately 10 mg of the surface portion of each tablet was scraped off and extracted by ultrasound for approximately 10 s in 200 µL of a 50 % water-containing acetonitrile solution. After centrifugation for the purpose of removing pharmaceutical additives, the obtained supernatant was separated for HPLC analysis.

3.4. HPLC analytical method

The following instruments and conditions were used in HPLC analysis: HPLC System: Shimadzu LC-10 series (LC-10AS, CBM-10A, CTO-10A, SIL-10AXL, SPD-M10 AVP). Column: Shiseido CAPCELL PAK C18 UG-120 (4.6 × 150 mm, 3.0 µm particles). Elution: A:B = 90:10 to 30:70 over 30 min; A = 1/5000 (trifluoroacetic acid/pure water), B = 1/5000 (trifluoroacetic acid/MeCN).
Flow rate = 1.0 mL/min, oven temperature = 40 °C, and detection at 254 nm and 220 nm.

3.5. NMR spectroscopy

NMR spectra were recorded on a JNM-ECZ400R/S1 spectrometer (JAPAN ELECTRON OPTICS LABORATORY CO., LTD, Tokyo, Japan), using CD_3OD solution with tetramethylsilane (TMS) as an internal standard.

3.6. Isolation of bromocriptine bulk powder

First, 50 tablets of Perlodel® 2.5 mg Tab. were pulverized in a mortar and then extracted by ultrasound for 5 min with 50 mL of MeOH. After removal of the insoluble precipitate, including formulation additives, by suction, the filtrate was concentrated under reduced pressure and purified by silica gel column chromatography (eluted with chloroform:MeOH = 20:1 to 10:1). The chemical structure of the isolated bromocriptine was identified through the comparison of its NMR spectral data with literature values (Maurer et al. 1982).

3.7. Spectral data of the major photoproduct (RT = 9.15 min in HPLC)

^{1}H NMR (in CD_3OD): δ 10.43 (1H, br s, $C_{9'}$-H), 9.99 (1H, br s, C_7-H), 8.97 (1H, s, C_4-H), 8.52 (1H, d, *J*= 8.7Hz, $C_{12'}$-H), 7.97 (1H, t, *J*= 7.8Hz, $C_{13'}$-H), 7.35 (1H, d, *J*= 7.3Hz, $C_{14'}$-H), 6.24 (1H, s, C_2-H), 4.96 (3H, br s, N_6-Me), 4.57 (1H, t, *J*= 6.4Hz, $C_{5'}$-H), 3.95 (1H, dd, *J*= 6.9 and 8.7Hz, $C_{11'}$-H), 3.56 (1H, dd, *J*= 5.0 and 9.1Hz, C5'-CH_2C<u>H</u>Me_2), 2.50–2.40 (1H, m, C2'-C<u>H</u>Me_2), 2.22–1.85 (8H,m, $C_{5'}$-C<u>H</u>-$_2$CHC<u>Me</u>$_2$, $C_{8'}$-2H, $C_{9'}$-2H, and $C_{10'}$-2H), 1.28 (3H, d, *J*= 6.4Hz, $C_{2'}$-CH<u>Me</u>$_2$), 1.14 (3H, d, *J*= 6.8Hz, $C_{2'}$-CH<u>Me</u>$_2$), 1.01 (3H, d, *J*= 6.4Hz, $C_{5'}$-CH_2CHC<u>Me</u>$_2$), and 0.97 (3H, d, *J*= 6.4Hz, $C_{5'}$-CH_2CHC<u>Me</u>$_2$) ppm.
^{13}C NMR (in CD_3OD): δ 168.6 ($C_{6'}$=O), 167.9 ($C_{3'}$=O), 167.3 (C_8-<u>C</u>O), 166.3 ($C_{12'}$), 150.7 (C_7), 143.9 (C_5), 142.2 ($C_{9'}$), 141.1 ($C_{10'}$), 137.4 ($C_{15'}$), 136.0 (C_2), 135.1 ($C_{13'}$), 130.5 ($C_{16'}$), 130.1 (C_8), 124.4 ($C_{11'}$), 117.8 ($C_{12'}$), 115.3 (C_4), 111.3 ($C_{14'}$), 93.0 (C_3), 79.5 ($C_{2'}$), 65.5 ($C_{11'}$), 54.6 ($C_{5'}$), 48.4 (N_6-<u>Me</u>), 47.4 ($C_{8'}$), 44.7 ($C_{5'}$-<u>C</u>H_2CHMe$_2$), 35.2($C_{2'}$-<u>C</u>HMe$_2$), 27.3 ($C_{9'}$), 26.2 ($C_{5'}$-CH_2<u>C</u>HMe$_2$), 23.1 ($C_{5'}$-CH_2CH<u>Me</u>$_2$), 23.0 ($C_{10'}$), 22.6 ($C_{5'}$-CH_2CH<u>Me</u>$_2$), 17.4 ($C_{2'}$-CH<u>Me</u>$_2$), and 16.4 ($C_{2'}$-CH<u>Me</u>$_2$) ppm.
IR (KBr): µ 3354, 3241, 1722, 1670, 1628, and 1539 cm^{-1}. (for bromocriptin: µ 3258, 1726, 1673, 1638, and 1547 cm^{-1}).
UV (MeOH) λ_{max} (ε): 460 (1.8×10^3), 314 (9.7×10^3), and 259 (2.3×10^4) nm. (for bromocriptin: 305 (ε 7.3×10^3) –325 nm).
MS: *m/z* 572.1 $[M+H]^+$ (calcd. for $C_{32}H_{37}N_5O_5$: MW=571.28).

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