Effects of Compression and Grinding on Chemical Stability of a Benzodiazepine Receptor Agonist

Megumi FUJITA,*[,a] Satoshi HIMI,[b] and Tetsurou HANDA[c]

[a] *Formulation Research and Development Laboratories, Dainippon Sumitomo Pharma Co., Ltd.; 1–5–51 Ebie, Fukushima-ku, Osaka 553–0001, Japan:* [b] *Research Department, Oriental Pharmaceutical and Synthetic Chemical Co., Ltd.; 2–5–4 Tsurumi, Tsurumi-ku, Osaka 538–0053, Japan: and* [c] *Graduate School of Pharmaceutical Sciences, Kyoto University; 46–29 Yoshida-Shimo-Adachi-cho, Sakyo-ku, Kyoto 606–8501, Japan.*

Received July 31, 2009; accepted October 24, 2009; published online October 27, 2009

SX-3228, 6-benzyl-3-(5-methoxy-1,3,4-oxadiazol-2-yl)-5,6,7,8-tetrahydro-1,6-naphthyridin-2(1H)-one, is a newly synthesized benzodiazepine receptor agonist intended to be developed as a tablet preparation. However, it was found that the drug substance was remarkably chemically unstable in tablet form compared to the powder mixture for tableting. Chemical destabilization due to compression also occurred in the drug substance alone. After investigating the cause of the destabilization, powder X-ray diffraction analysis showed that the crystallinity of the drug substance decreased depending on the extent of mechanical treatments such as compression and grinding. In thermal analysis it became evident that the exothermic peaks due to degradation clearly broadened and shifted toward lower temperatures by these mechanical treatments. It was then revealed that the degradation temperature decreased and the amount of degradation products after storage increased with decreasing crystallinity, even though there was little change in the amount of degradation products shortly after mechanical treatments. These results demonstrated that the drug substance became chemically unstable with decreasing crystallinity. It was proved that chemical instability of the drug substance in the tablet preparation was due to decreasing crystallinity caused by compression. It would therefore be difficult to produce chemically stable tablets containing this compound using a conventional manufacturing process. Tablets for this compound should be prepared without mechanical treatments such as compression and grinding.

Key words crystallinity; stability; compression; grinding; degradation

A tablet preparation is one of the most patient-preferred dosage forms of a pharmaceutical product because it is easy to handle and easy to take. Tablets offer precise dosing each time and coatings for various purposes are also possible. For these reasons a tablet preparation is often the preferred choice dosage form for new drug substances. Tablets are generally prepared by compression methods using high compression pressure between approximately 100 and 200 MPa, and it is well known that drug substances are exposed to various factors such as pressure, friction and heat during compression.

There are several reports concerning the effects of compression or grinding on the crystallinity of drug substances and, for example, it has been reported that the crystallinity of cephalexin, α-tricalcium phosphate and chloramphenicol palmitate decreased by compression or grinding with a ball mill.[1—3] Drug substances are sometimes modified from crystalline solids to the amorphous state in order to improve their solubility, even though it is generally known that amorphous materials have poor chemical stability compared to crystalline materials.[4,5] However, literature is limited that clearly describes the relationship between crystallinity and chemical stability of drug substances mechanically-treated by compression and grinding.[6—8]

SX-3228, 6-benzyl-3-(5-methoxy-1,3,4-oxadiazol-2-yl)-5,6,7,8-tetrahydro-1,6-naphthyridin-2(1H)-one (Fig. 1A), is a benzodiazepine receptor agonist that was newly synthesized at Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan), and was targeted for use as a hypnotic drug.[9—13] This compound is a non-benzodiazepine derivative with demonstrable selectivity for the benzodiazepine ω_1 receptor subtype.[9—13] Although intended to be developed as a tablet preparation, the drug substance turned out to be chemically unstable in tablet form even though its stability was comparatively good in the powder mixture for tableting.

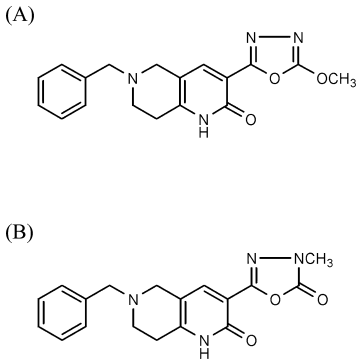

Fig. 1. Chemical Structure of 6-Benzyl-3-(5-methoxy-1,3,4-oxadiazol-2-yl)-5,6,7,8-tetrahydro-1,6-naphthyridin-2(1H)-one (SX-3228, Molecular Formula: $C_{18}H_{17}N_4O_3$, Molecular Weight: 338.37) (A) and Methyl-Rearranged SX-3228 (B)

The objective of this study was to clarify the cause of poor chemical stability of the drug substance in the tablet preparation using powder X-ray diffraction measurement, thermal analysis and HPLC analysis.

Experimental

Materials SX-3228 was synthesized at Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan.

Pharmaceutical Excipients: Japanese Pharmacopoeia (fifteenth edition) grade excipients were used. Lactose (Tablettose® 80) (Meggle GmbH, Wasserburg, Germany), cornstarch (Nihon Shokuhin Kako Co., Ltd., Tokyo, Japan), low-substituted hydroxypropylcellulose (L-HPC, LH-11) (Shin-Etsu Chemical Industry Co., Ltd., Tokyo, Japan), magnesium stearate (animal origin) (Taihei Chemical Industrial Co., Ltd., Osaka, Japan) and light anhy-

* To whom correspondence should be addressed. e-mail: megumi-fujita@ds-pharma.co.jp

drous silicic acid (LASA, Aerosil® 200) (Nippon Aerosil Co., Ltd., Tokyo, Japan) were used.

Reagents: Methanol of HPLC grade and all other reagents of analytical grade were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Preparation and Storage of SX-3228 Tablets SX-3228 tablet formulation contained the drug substance 5 mg, lactose 55 mg, cornstarch 23 mg, L-HPC 15 mg, magnesium stearate 1 mg and LASA 1 mg (total weight: 100 mg/tablet). The tablets were prepared by a direct compression method with a pressure of 200 MPa using a single punch tableting machine (2B, Kikusui Seisakusho Ltd., Kyoto, Japan) fitted with a 6.5 mm diameter punch. The drug substance alone was also compressed in the same way. The intact and compressed drug substance, the powder mixture for tableting and the tablets were stored in open glass bottles at 80 °C and 60 °C/75% relative humidity (RH) for 14 d in order to evaluate their chemical stability.

Mechanical Treatment and Storage of SX-3228 Drug Substance SX-3228 drug substance was first mechanically treated by either compression or grinding. Each 200 mg of intact drug substance was compressed with pressures of 20, 50 and 200 MPa using the same machine as for tablet preparation, fitted with an 8.0 mm diameter punch. As for grinding, about 5 g of the intact drug substance was ground with an agate centrifugal ball mill (P-7, FRITSCH GmbH, Idar-Oberstein, Germany) for 1, 3 and 60 min. The grinding conditions were as follows: capacity of each container, 45 ml; diameter and number of balls, 15 mm and 7; rotation speeds of disk and containers, 600 rpm. The intact and mechanically-treated drug substances were subjected to powder X-ray diffraction analysis and thermal analysis and the appearance of crystals was observed.

The drug substance was also compressed with pressures of 50 and 200 MPa and ground for times varying between 3 to 60 min in order to investigate the relationship between relative crystallinity and degradation temperature or chemical stability. Chemical stability was evaluated after storage in open glass bottles at 70 °C and 50 °C/75% RH for 14 d.

Observation of Crystals Appearance of crystals was observed using a scanning electron microscope (SEM) (JSM-5900LV, JEOL Ltd., Tokyo, Japan) in a high vacuum mode after platinum evaporation coating.

Powder X-Ray Diffraction Analysis Powder X-ray diffraction was performed with an X-ray diffractometer (XRD6100, Shimadzu Corp., Kyoto, Japan or RINT1100, Rigaku Corp., Tokyo, Japan). The measurement conditions were as follows: target Cu, voltage 30 kV, current 20 mA, receiving slit 0.15 mm, scanning speed 3°/min, investigation range $2\theta=10$—40°. Relative crystallinity was evaluated by a reflection intensity method using lithium fluoride (LiF) that had a diffraction intensity at $2\theta=38.4°$ as an internal standard. The relationship of the diffraction intensity ratios of the three main peaks of SX-3228 drug substance ($2\theta=14.2°$, 16.6°, 18.4°) to the peak of LiF and the weight ratios of the drug substance and LiF mixture was examined and there was a linear relationship between these values when the weight ratio of LiF in the mixture was 20% or larger. Therefore, relative crystallinity was expressed as a percentage of an average of the three peak intensity ratios of the compressed or ground drug substance to the average of the intact drug substance, using mixed powder that consisted of 20% LiF and 80% drug substance.

Thermal Analysis Thermal behavior was determined using a differential scanning calorimetry (DSC) instrument (DSC2920, TA Instruments, Inc., New Castle, DE, U.S.A., or TAS100, Rigaku Corp., Tokyo, Japan). The measurement conditions were as follows: sample weight, about 2 mg; heating rate, 5 °C/min.

Evaluation of Chemical Stability Chemical stability was evaluated with an HPLC system consisting of an LC-10AS pump, a CTO-10A column oven, an SPD-10A UV–visible detector, an SCL-10AVP system controller, an SIL-10AXL auto injector and a CLASS-VP data system (Shimadzu Corp., Kyoto, Japan). A 150 mm×5 mm internal diameter column packed with octadecyl silica gel of 5 μm particle size, Develosil ODS-5 (Nomura Chemical Co., Ltd., Aichi, Japan) was utilized at 40 °C. The mobile phase consisted of 65% 0.01 mol/l acetate buffer (pH 4.0) and 35% methanol and the flow rate was set at about 1.0 ml/min. Retention time of SX-3228 was around 11.0 min. SX-3228 and its degradation products were extracted from around 20 mg of the drug substance or from 4 tablets with 50 ml of purified water–methanol (2 : 3) and 5 μl of the extract was injected. The peaks of the degradation products were identified based on the retention time of the standard materials. The drug substance and its degradation products were detected at a wavelength of 342 nm and degradation product amounts were expressed as a peak area percentage of the sum of SX-3228 and its degradation products.

Results and Discussion

Chemical Destabilization by Compression of SX-3228 Drug Substance Chemical stability of SX-3228 in tablet form was evaluated. Figure 2 presents the HPLC profile of SX-3228 tablets after storage at 80 °C for 14 d as a typical chromatogram. The results in Table 1 showed that the total amount of degradation products increased from 0.9 to 5.4 and 11.9% during the 14 d storage at 80 °C and 60 °C/75% RH respectively. The increases were much larger than those in the powder mixture for tableting, which were from 0.7 to 2.2 and 2.0%, respectively. The effects of compression on chemical stability on the drug substance alone were then examined and similar results to those for the tablets and powder mixture for tableting were obtained, as presented in Table 1. The adverse impact of compression was especially large under the high-humidity condition and the increase in the total amount of degradation products during storage in the drug substance and the powder mixture was 18-fold and 11-fold respectively. The cause of the chemical destabilization by compression shown by these results was then investigated.

Effect of Compression and Grinding on Relative Crystallinity of SX-3228 Drug Substance Appearance of crystals in the intact and mechanically-treated SX-3228 drug substance was checked by SEM. Whereas the intact drug substance contained columnar crystals of around 15 to 150 μm in length, these were observed to be clearly broken in the compressed and ground drug substance as shown in Fig. 3. Powder X-ray diffraction profiles in Fig. 4 indicated that the crystalline form was not changed by these mechanical treatments because the peak positions of the compressed and ground drug substance were almost the same as those of the

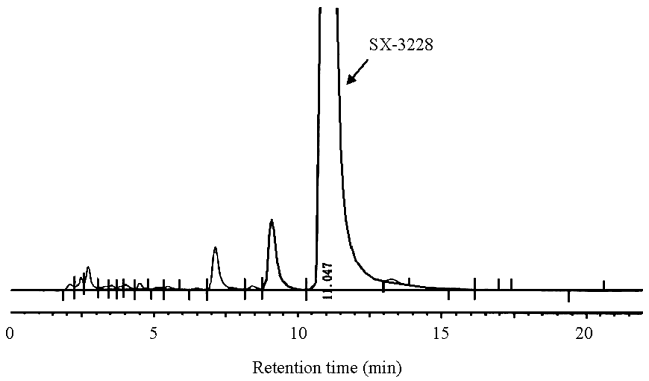

Fig. 2. HPLC Profile of SX-3228 Tablets after Storage at 80 °C for 14 d

Table 1. Effect of Compression on Chemical Stability of SX-3228 in the Drug Substance and Powder Mixture (Compression Pressure: 200 MPa, Stored in Open Bottles)

Sample	Total amount of degradation products (%)		
	Initial	After 14 d at 80 °C	After 14 d at 60 °C/75% RH
Intact drug substance	0.73±0.02	1.89±0.05	1.17±0.02
Compressed drug substance	0.78±0.01	3.25±0.04	8.69±0.02
Powder mixture for tableting	0.73±0.02	2.23±0.00	1.96±0.04
Tablets	0.85±0.03	5.44±0.07	11.91±0.39

The data are expressed as means±standard deviations, $n=3$.

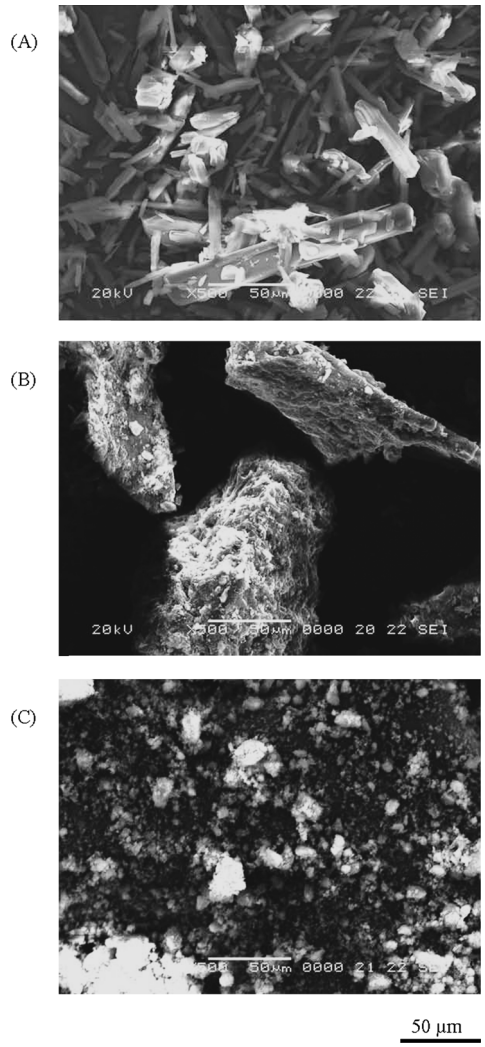

Fig. 3. SEM Photographs of the Intact and Mechanically-Treated SX-3228 Drug Substance

(A) Intact, (B) compressed at 200 MPa, (C) ground for 60 min.

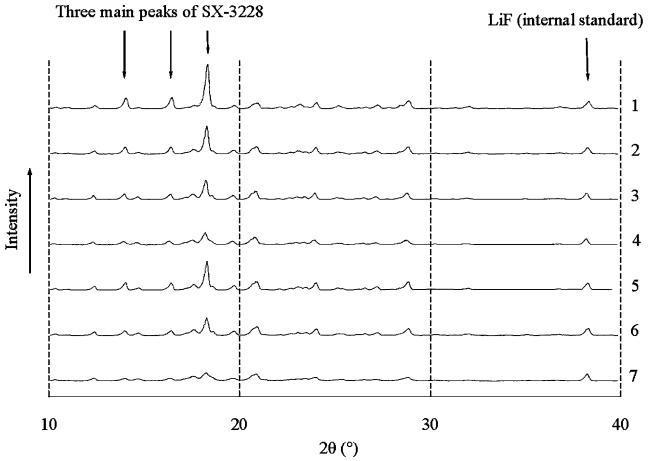

Fig. 4. Change in X-Ray Diffraction Profiles of SX-3228 Drug Substance by Various Mechanical Treatments (SX-3228 : LiF = 4 : 1)

1, intact; 2, compressed at 20 MPa; 3, compressed at 50 MPa; 4, compressed at 200 MPa; 5, ground for 1 min; 6, ground for 3 min; 7, ground for 60 min.

Table 2. Change in Relative Crystallinity and Exothermic Peak Temperature in DSC (Degradation Temperature) of SX-3228 Drug Substance by Various Mechanical Treatments

Sample	Relative crystallinity (%)	Exothermic peak temperature in DSC (degradation temperature) (°C)
Intact	100.0±6.9	179.09±0.92
Compressed at 20 MPa	68.1±5.7	175.99±0.30
Compressed at 50 MPa	45.6±1.4	173.92±0.47
Compressed at 200 MPa	32.4±0.7	171.03±0.01
Ground for 1 min	61.7±0.5	176.62±0.10
Ground for 3 min	40.0±1.6	172.59±0.38
Ground for 60 min	21.2±0.4	160.44±0.56

The data are expressed as means±standard deviations, $n=3$.

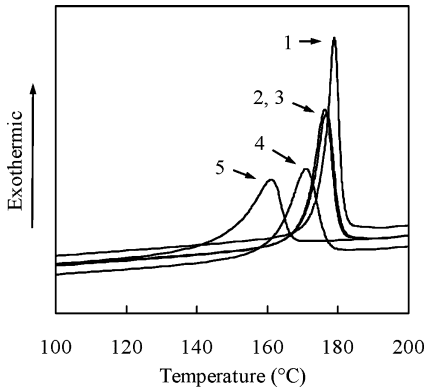

Fig. 5. Change in DSC Curves of SX-3228 Drug Substance by Various Mechanical Treatments

1, intact; 2, compressed at 20 MPa; 3, ground for 1 min; 4, compressed at 200 MPa; 5, ground for 60 min.

intact drug substance. The compressed and ground drug substance, however, appeared to be low in diffraction intensity compared to the intact drug substance as presented in Fig. 4, and so the relative crystallinity of these samples was calculated. The results revealed that the relative crystallinity of the drug substance compressed with pressures of 20, 50 and 200 MPa was about 68, 46 and 32% respectively and that of the drug substance ground in a centrifugal ball mill for 1, 3 and 60 min was about 62, 40 and 21% respectively as listed in Table 2. From these results, we surmised that the crystallinity of the drug substance decreased markedly and that about 32 to 79% of crystals in the intact drug substance became noncrystalline by mechanical treatments.

Thermal Analysis of the Intact and Mechanically-Treated SX-3228 Drug Substance The DSC curves of the intact, compressed and ground SX-3228 drug substance are presented in Fig. 5. The exothermic peak was observed at around 179 °C in the intact drug substance (Table 2) and its appearance changed from light yellowish-white powder to black semisolid state at this temperature. Retention time of the semisolid material was around 9.1 min and the material was identified as a methyl-rearranged product of the drug substance based on the retention time of the standard material of the methyl-rearranged product by HPLC analysis. Figure 1B shows the chemical structure of the methyl-rearranged product. The product was considered to be produced through a Chapman-like thermal rearrangement of the methyl group from the chemical structure of SX-3228 and the reaction product (Figs. 1A, B).[14—16)]

The exothermic peak temperature of the drug substance compressed with pressures of 20, 50 and 200 MPa was about 176, 174 and 171 °C respectively and that of the drug substance ground in a centrifugal ball mill for 1, 3 and 60 min was around 177, 173 and 160 °C respectively as shown in Table 2. These results showed the peak shifted toward lower temperatures and was clearly broadened by the mechanical treatments.

Relationship between Relative Crystallinity and Degradation Temperature of SX-3228 Drug Substance Figure 6A shows the relationship between relative crystallinity and degradation temperature (the exothermic peak temperature in DSC) of the intact and mechanically-treated SX-3228 drug substance. With various degrees of treatments of compression at 50 and 200 MPa and grinding for 3 to 60 min, the relative crystallinity decreased from 100% to between 58 and 25% and the degradation temperature was lowered from 180 °C to between 175 and 158 °C. The degradation temperature decreased with crystallinity, and the lower the crystallinity, the larger the decrease in the degradation temperature. These results suggest that the drug substance became chemically unstable with decreasing relative crystallinity.

Relationship between Relative Crystallinity and Chemical Stability of SX-3228 Drug Substance Figure 6B summarizes the relationship between relative crystallinity and the total amount of degradation products after storage at 70 °C and 50 °C/75% RH of the intact and mechanically-treated SX-3228 drug substance. Shortly after compression and grinding, the total amount of degradation products was nearly 0% in all samples that possessed relative crystallinity between 100 and 25% due to varying levels of compression and grinding (initial data in Fig. 6B). However the amount of degradation products increased during the 14 d storage under both conditions and inversely correlated with the crystallinity when relative crystallinity was lower than 60%. At a relative crystallinity of around 25%, the total amount of degradation products increased to approximately 9 and 24% after storage at 70 °C and 50 °C/75% RH, respectively. With a relative crystallinity of higher than 60%, just a slight increase was observed in the amount of degradation products after storage. The main degradation product was the methyl-rearranged product presented in Fig. 1B. The increase in the amount of degradation products of the compressed and ground drug substance was much larger under the high-humidity condition than just the high-temperature condition in agreement with the results shown in Table 1. These results demonstrated that the drug substance became chemically unstable with decreasing crystallinity and that chemical instability of the drug substance in the tablet preparation indicated in Table 1 was due to a decrease in crystallinity caused by compression.

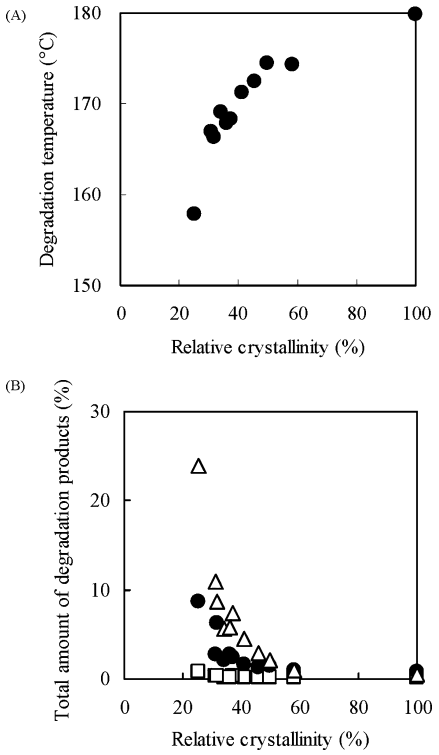

Fig. 6. (A) Relationship between Relative Crystallinity and Degradation Temperature of the Intact and Mechanically-Treated SX-3228 Drug Substance and (B) Relationship between Relative Crystallinity and the Total Amount of Degradation Products of the Intact and Mechanically-Treated SX-3228 Drug Substance

□, initial; ●, after 14 d at 70 °C (in open bottles); △, after 14 d at 50 °C/75% RH (in open bottles).

Conclusion

It was found that the SX-3228 drug substance was remarkably chemically unstable in tablet preparation compared to the powder mixture for tableting. Chemical destabilization caused by compression also occurred in the drug substance alone. After investigating the cause of the destabilization, it became evident that the crystallinity of the drug substance decreased depending on the extent of mechanical treatments such as compression and grinding. It was then revealed by thermal analysis that the degradation temperature was lowered and the amount of degradation products after storage increased with decreasing crystallinity. These results demonstrated that the drug substance became chemically unstable with decreasing crystallinity. It was proved that chemical instability of the drug substance in the tablet preparation was due to decrease in crystallinity by compression. It would therefore be difficult to produce chemically stable tablets containing this compound using a conventional manufacturing process. Tablets for this compound should be prepared without mechanical treatments such as compression and grinding.

References

1) Kaneniwa N., Imagawa K., Otsuka M., *Chem. Pharm. Bull.*, **33**, 802—809 (1985).
2) Camire C. L., Gbureck U., Hirsiger W., Bohner M., *Biomaterials*, **26**, 2787—2794 (2005).
3) Otsuka M., Kaneniwa N., *J. Soc. Powder Technol. Jpn.*, **23**, 63—67 (1986).
4) Terada K., Kitano H., Yoshihashi Y., Yonemochi E., *Pharm. Res.*, **17**, 920—924 (2000).
5) Yoshihashi Y., Kitano H., Yonemochi E., Terada K., *Int. J. Pharm.*, **204**, 1—6 (2000).
6) Matsunaga Y., Bando N., Yuasa H., Kanaya Y., *Chem. Pharm. Bull.*, **44**, 1931—1934 (1996).
7) Mimura H., Kitamura S., Okamoto Y., Yasuda T., *Drug Stability*, **1**, 34—39 (1995).
8) Otsuka M., Kaneniwa N., *Int. J. Pharm.*, **62**, 65—73 (1990).
9) Ohta T., *Jpn. J. Psychopharmacol.*, **16**, 161—170 (1996).
10) Sanger D. J., *Behav. Pharmacol.*, **8**, 287—292 (1997).
11) Griebel G., Perrault G., Sanger D. J., *J. Psychopharmacol.*, **12**, 356—365 (1998).
12) Griebel G., Perrault G., Tan S., Schoemaker H., Sanger D. J., *Behav.*

Pharmacol., **10**, 483—495 (1999).
13) Alvarino F., Monti J. M., Jantos H., Monti D., *Braz. Med. Biol. Res.*, **32**, 1007—1014 (1999).
14) Dessolin M., Golfier M., *J. Chem. Soc., Chem. Commun.*, **1986**, 38—39 (1986).
15) Venugopalan P., Venkatesan K., Klausen J., Novotny-Bregger E., Leumann C., Eschenmoser A., Dunitz J. D., *Helv. Chim. Acta*, **74**, 662—667 (1991).
16) Dessolin M., Eisenstein O., Golfier M., Prangé T., Sautet P., *J. Chem. Soc., Chem. Commun.*, **1992**, 132—134 (1992).