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Note

Photodecomposition of Moclobemide on a silica gel thin-layer chromatographic plate

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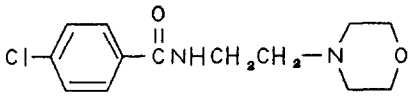
Moclobemide [*p*-chloro-N-(2-morpholinoethyl)benzamide] (MBD), a monoamine oxidase (MAO) inhibitor, is an antidepressant and has also recently been developed as a nootropic drug.



During our thin-layer chromatographic (TLC) study of MBD an unknown spot was occasionally detected on a silica gel plate by UV irradiation (254 nm) or by spraying with 4,4′-tetramethyldiaminodiphenylmethane (TDM) reagent after chlorination. We needed to find out why this spot had appeared; therefore, we began to study the stability of the sample solution, possible contamination, the quality of the TLC plate and the influence of light or air on the sample.

In the presence of diffuse daylight, MBD adsorbed on the silica gel of the TLC plate was found to give an unknown spot, which was later determined to be a photodecomposition product of MBD. The production of an artifact or photodegradation of MBD during TLC has not been investigated before. However, photochemical reactions of compounds adsorbed on silica gel have been reported for Vitamin K_3[1], 3-alkyl-1-phenyltriazine[2], stilbene[3], dipyridamol[4] and pyrazolidine-3,5-diones[5].

The aim of the present study was to investigate the cause of the decomposition of MBD and to identify its decomposition products in order to develop adequate TLC conditions for MBD. In this paper, an high-performance liquid chromatographic (HPLC) method to identify photodegradation products of MBD is also described.

EXPERIMENTAL

Materials and reagents

Moclobemide and its N-oxide derivative were from F. Hoffmann-La Roche (Basle, Switzerland). All reagents were of analytical grade. All materials and reagents were used without further purification.

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Chromatographic procedures

Silica gel 60 F_{254} pre-coated plates (E. Merck, F.R.G.) were used for TLC. The plates were developed with chloroform–methanol–water (13:6:1, v/v/v; system I) or ethyl acetate–ethanol–28% ammonia (8:2:1, v/v/v; system II).

In addition to normal-phase TLC, reversed-phase TLC was performed by using a $KC_{18}F$ precoated plate (Whatman, U.K.) with a mobile phase methanol–water (3:2, v/v). All plates were air dried, and the spots were detected by irradiation with short-wavelength ultraviolet light (254 nm). Our HPLC system consisted of a Model CCPD pump and a Model UV-8000 detector set at 235 nm (Tosoh, Japan) and a 7125 injector (Rheodyne, U.S.A.), and the following chromatographic systems were employed: (A) an ODS-80 column (15 cm x 4.6 mm I.D.) (Tosoh) with methanol–water (3:2, v/v) as the eluent at a flow-rate of 0.8 ml/min for preparative HPLC. (B) an Inertsil-ODS column (15 cm × 4.6 mm I.D.) (Gasukuro Kogyo, Japan) with acetonitrile–0.1% triethylamine solution adjusted to pH 6.0 with 50% phosphoric acid (1:3, v/v) as the eluent at a flow-rate of 1.2 ml/min for identification of unknown compounds.

Procedure for photodegradation of moclobemide on the TLC plate

The plates with 100 μg of MBD were exposed for several hours to various sources of light by using a fademeter (Model CF-20S; Shimadzu, Japan) with a wavelength below 275 nm, a near-UV lamp (FL20S.BI; Toshiba, Japan) with a spectral distribution between 290 and 390 nm, an ordinary white fluorescent lamp (400–700 nm) and diffuse daylight or a UV lamp (254 nm). Each plate was developed with solvent system I in order to confirm whether degradation had occurred.

Separation and purification

A l-ml volume of 10% (w/v) MBD in methanol was streaked on the silica gel TLC plate. The plate was exposed to diffuse daylight for 3 h and then developed using solvent system I. A narrow band at R_F 0.37 from four plates was scraped off, and the silica was extracted with 25 ml of methanol. After centrifugation at *ca.* 1600 *g*, the residual silica was reextracted with methanol. The pooled methanol extracts were filtered by using a membrane filter (0.25 μm, hydrophobic; Toyo Roshi, Japan) and then evaporated to dryness under reduced pressure. The residue was dissolved in 300 μl of methanol and the solution was subjected to our HPLC system A. Samples corresponding to the predominant peak with a retention time of about 4.4 min were collected. About 0.4 mg of material were obtained after drying the pooled fractions.

Structural elucidation

NMR spectra were recorded with a 400-MHz Model GX-270 spectrometer (JEOL, Japan). Low- and high-resolution mass spectra were taken by direct injection of the sample with a Model DX-300 spectrometer (JEOL).

RESULTS AND DISCUSSION

MBD has been reported to be stable against heat, humidity and light[6]; however, during our TLC study of this compound, an unknown spot was occasionally detected by UV irradiation (254 nm) or by spraying with TDM reagent after chlo-

rination. In order to explain the cause of this phenomenon, the stability of the sample solution, possible contamination, the quality of the TLC plate and the effect of light or air on MBD were investigated.

Among the various sources of light described in the procedure for photodegradation section, the carbon arc fademeter and diffuse daylight gave the unknown spot in the TLC chromatogram. Thus we thought light, especially with wavelength below 275 nm, was the main factor in the decomposition of MBD. The photodecomposition was dependent on the difference in light sources, though the same lot of TLC plate was used.

An unknown spot was also detected when MBD was applied to a silica gel plate without the fluorescent additive (silica gel 60, precoated, Merck) or a plate previously developed with methanol and dried, under diffuse daylight which had passed through glass window-panes.

We confirmed no decomposition of MBD by spotting and developing a chromatogram in the dark, indicating that photolysis plays a decisive role.

These findings mean that the quality of the silica gel plate is not responsible for the decomposition of MBD, in contrast with the case reported by Macek[7].

Upon exposure to the carbon arc fademeter, no decomposition of MBD was observed when dissolved in methanol, acetonitrile and chloroform which is said to be sensitive to light, while the formation of the N-oxide derivative was more or less detected for the MBD in dichloromethane, dichloroethane, acetone and dioxane solutions.

No photodecomposition of MBD was found in the solid state, in which the particle size is about 14 μm, when exposed to direct rays from the sun on the roof of our laboratory.

In addition, during experimentation, the photodecomposition of MBD on a

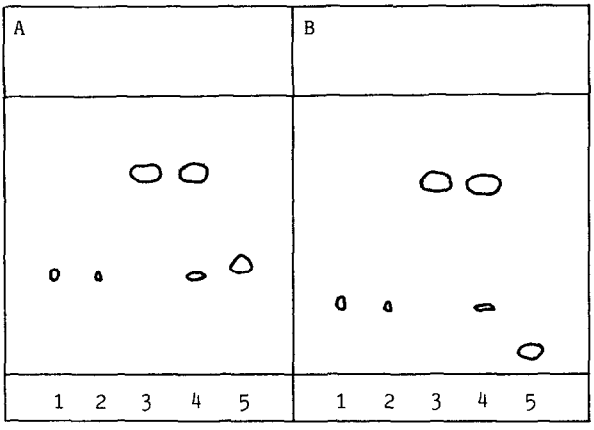


Fig. 1. Separation of the sample and standards on a silica gel TLC plate. (A) Solvent system, chloroform–methanol–water (13:6:1); (B) solvent system, ethyl acetate–ethanol–28% ammonia (8:2:1). Spots: 1 = isolated decomposition product (*ca.* 4 μg); 2 = authentic N-oxide of MBD (2 μg); 3 = MBD (100 μg); 4 = exposed MBD (100 μg); 5 = *p*-chlorobenzoic acid (10 μg). Samples 1, 2, 3 and 5 were applied to the plate after spotting MBD and exposure to diffuse daylight passing through a glass window-pane for 3 h. Detection: irradiation with UV light (254 nm).

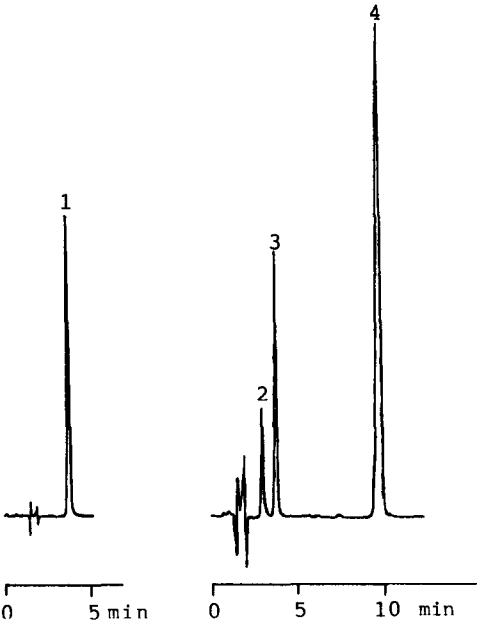


Fig. 2. Chromatograms of the isolated photodecomposition product (A) and a mixture of standards (B). Peaks: 1 = isolated photodecomposition product; 2 = *p*-chlorobenzoic acid; 3 = authentic N-oxide of MBD; 4 = MBD. Conditions: column, 150 mm × 4.6 mm I.D. Inertsil-ODS (5 μm); mobile phase, acetonitrile–0.1% triethylamine solution adjusted to pH 6.0 with phosphoric acid (1:3, v/v); flow-rate 1.2 ml/min; room temperature; UV detection at 235 nm.

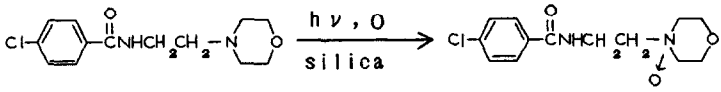
TLC plate proved to be irreproducible, possibly because it was influenced by the day-to-day differences in the amount of diffuse daylight that reached the plate.

The structure of the compound isolated was identified by subjecting it to low- and high-resolution mass spectroscopy as well as to 400-MHz NMR analysis. The molecular weight of the sample was shown to be 284 by low-resolution mass spectroscopy, and the elemental composition of the molecular fragment ion was found to be $C_{13}H_{17}ClN_2O_3$ by high-resolution mass spectrometry. Our results show that the degradation product contains one more oxygen atom compared with MBD. Proton NMR spectra for MBD and its decomposition product in C^2HCl_3 showed that the chemical shifts of protons in the morpholine moiety of the decomposition product are different from those of MBD, indicating that an N-oxide had been formed. The structural assignments for the decomposition product were confirmed by comparing it to an authentic N-oxide of MBD.

Furthermore, the decomposition product isolated was chromatographed together with an authentic N-oxide of MBD by TLC solvent systems I and II as well as by HPLC system B. As shown in Figs. 1 and 2, the chromatographic behaviour of the decomposition product was identical with that of the synthetic N-oxide of MBD. *p*-Chlorobenzoic acid, the main degradation product of MBD, can also be separated by TLC and HPLC (Figs. 1 and 2). Accordingly, these analytical conditions can be used to monitor the stability of MBD.

The reported decomposition by photooxidation occurs when MBD is adsorbed

on the silica gel of the TLC plate and exposed to light with a wavelength of less than 275 nm. As described previously[8] the reaction is thought to result from the excitation of silica by UV light, and then the oxygen excited by the silica reacts with MBD:



This assumption is probably supported by the fact that a remarkable decrease in the formation of N-oxide was observed when MBD was spotted on an ODS TLC plate and exposed to diffuse daylight. However, different degradation products from N-oxide were produced on the ODS plate. The chemical structure of the new degradation products is now under investigation. Since the decomposition product of MBD is an artifact formed by the interaction of light and MBD on the silica gel TLC plate, samples should be applied promptly and protected from intens diffuse daylight during the TLC procedure.

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