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Photochemical stability of dothiepin in aqueous solutions

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Summary

Dothiepin, a tricyclic antidepressant, is slowly decomposed in aqueous solutions exposed to light. Artificial radiation from a high-pressure mercury lamp caused opening of the 7-ring and rearrangement of the molecule into a benzothiophene derivative. The main decomposition products in daylight were sulphoxides, with some other compounds formed in trace amounts. Glass capillary gas chromatography with non-polar OV-101 as liquid phase proved to be an accurate and reproducible procedure for monitoring of the isomerization and degradation of dothiepin.

Introduction

Dothiepin hydrochloride (Prothiaden)(3-dibenzo[b,e]thiepin-11(6*H*)-ylidene-*N*, *N*-dimethyl-1-propanamine hydrochloride) is a tricyclic antidepressant with actions and uses similar to those of amitriptyline. The drug is used predominantly as the *trans* isomer, although there is no substantial difference in the central nervous system (CNS) activity of the two geometric isomers (Rajšner et al., 1969).

Dothiepin is a light-sensitive substance and should be stored protected from light (BP, 1988; Reynolds and Prasad, 1982). Under the influence of light it undergoes isomerization and on longer exposure several degradation products are formed (Li Wan Po and Irwin, 1980). One of the products has been identified as dothiepin *S*-oxide (private communication). Upon oral administration dothiepin is extensively metabolized by demethylation and oxidation (Crampton et al., 1978), mainly to dothiepin *S*-oxide, northiaden (= monodesmethyldothiepin) and northiaden *S*-oxide. Recently, also dothiepin and northiaden sulphones have been isolated and identified among the metabolites (Kawahara et al., 1987).

Specific analytical techniques are required for metabolic and degradation studies. Widely used methods include gas chromatography (GC) with nitrogen-sensitive (Dawling and Braithwaite, 1978) or mass spectrometric (Crampton et al., 1980; Maguire et al., 1981) detection and high-performance liquid chromatography (HPLC) with UV (Li Wan Po and Irwin, 1979; Šlais and Šubert, 1980; Yu et al., 1986; Jane et al., 1985) or electrochemical (Jane et al., 1985; Shibanoki et al., 1987) detection. HPLC allows simultaneous determina-

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tion of the geometric isomers (Li Wan Po and Irwin, 1979, 1980; Šlais and Šubert, 1980).

In the present study, aqueous solutions of dothiepin hydrochloride were subjected to radiation from a high-pressure mercury lamp or to daylight and degradation products were identified by various physicochemical methods. The degradation rate of the drug was monitored by a glass capillary GC method developed for the purpose.

Materials and Methods

Materials

Dothiepin hydrochloride (I) was kindly supplied by The Boots Company Ltd. (Nottingham, Great Britain). The drug consisted mainly of the *trans* isomer (2% of *cis* isomer checked by GC). Dothiepin sulphoxide (3-dibenzo[b,e]thiepin-11(6*H*)-ylidene-5-oxide-*N*,*N*-dimethyl-1-propanamine) and dothiepin sulphone (3-dibenzo[b,e]-thiepin-11(6*H*)-ylidene-5,5-dioxide-*N*,*N*-dimethyl-1-propanamine) were prepared by oxidizing the parent compound with sodium metaperiodate (Tammilehto et al., 1986). The free bases were converted to hydrogen maleate or hydrochloride salts. The identity and purity of the reference substances were verified by melting point, TLC, and UV, IR and MS spectra. All other reagents and solvents were of analytical grade.

Apparatus and methods

Elemental analyses were performed by the Ilse Beetz Microanalytical Laboratory (Kronach, F.R.G.). The melting points were determined with an Electrothermal digital melting point apparatus and are uncorrected. The UV spectra were recorded in ethanol with a Unicam SP 500 spectrometer, and the IR spectra with a Unicam SP 1000 infrared spectrometer (KBr disc). ^{1}H and ^{13}C NMR spectra were run on a Jeol JNM-FX 200 FT spectrometer with tetramethylsilane as internal standard. Mass spectra were recorded on a Jeol JMS-D 100 spectrometer at 75 eV with direct inlet. The solvent systems and R_f values of the TLC experiments are listed in Table 1. The spots were detected under UV light (254 nm). A high-pressure mercury lamp (Original Hanau TQ 150) was used in the irradiation studies. GC analyses were performed with a Fractovap 4200 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame-ionization detector and connected to a Hewlett-Packard 3380 A peak integrator. The glass capillary column (length 20 m, i.d. 0.3 mm) was coated with OV-101. The operating temperatures were: column, 215 °C; injection port and detector, 245 °C. Helium was employed as the carrier gas, and the splitting technique was used in all experiments.

TABLE 1

R_f values of dothiepin and its photodecomposition products

Compound	Solvent system			
	(A)	(B)	(C)	(D)
Dothiepin	0.66	0.47	0.43	0.61
Dothiepin sulphoxide	0.33	0.10	0.13	0.37
Unknown sulphoxide	0.42	0.15	0.19	0.44
Dothiepin sulphone	0.49	0.23	0.15	0.46
Compound III	0.80	0.68	0.74	0.63

Silica gel 60 F_{254} plates (20 × 20 cm), migration distance 15 cm. (A), toluene–ethyl acetate–diethylamine (10 : 2 : 1). (B), toluene–dioxane–ammonia 25% (6 : 3.5 : 0.5)(upper phase). (C), toluene–ethanol–water (10 : 10 : 1). (D), 1-butanol–acetic acid–water (6 : 2 : 2).

Photodegradation of dothiepin hydrochloride. Aliquots (2 ml) of the 0.2–1.0% dothiepin hydrochloride solutions, prepared in 0.1 M hydrochloric acid or acetate buffer at pH 5.6 (Brezina and Zuman, 1958), were charged into clear 10-ml glass ampoules. The ampoules were exposed to radiation from a high-pressure mercury lamp or to daylight on a window-sill. At appropriate time intervals the solutions were made alkaline with 1 ml of 1 M sodium hydroxide and extracted with chloroform (5 + 4 ml). The chloroform extracts were filtered through anhydrous sodium sulphate into 10-ml volumetric flasks and diluted to volume in chloroform. For the GC analysis 1.0–3.0 ml of the extract and 1.0 ml of the internal standard solution (chlorprothixene hydrochloride 2 mg/ml in chloroform) were diluted to exactly 5 ml, and 0.5–1.0 µl of this solution was injected into the gas chromatograph. All analyses were carried out in triplicate.

For the quantification of dothiepin, a calibration graph was constructed in the range 0.06–0.6 mg/ml by plotting peak area ratios of dothiepin to the internal standard versus dothiepin concentration.

Photodegradation product: 2-(2-dimethylaminoethyl)-3-(2-methylphenyl)-benzo[b]thiophene (III). A 0.5% dothiepin hydrochloride solution in 0.1 M hydrochloric acid (100 ml) was irradiated by Hg lamp for 9 h, whereafter the solution was extracted twice with chloroform. The chloroform extracts were washed successively with 0.1 M HCl and water, dried (Na_2SO_4), and evaporated to dryness in vacuo. The residue was washed with small portions of acetone, and dried in an exsiccator. Slightly brown powder, m.p. 202–206 °C (dec.). *Anal.*: Calculated for $C_{19}H_{21}NS \cdot HCl$: C, 68.76; H, 6.68; N, 4.22; S, 9.66. Found: C, 68.33; H, 6.95; N, 4.30; S, 9.49. The R_f values in different solvent systems are listed in Table 1. UV λ_{max}(log ϵ): 231(4.52), 262(3.99), 292(3.59), 300(3.61) nm. IR ν_{max}: 3060, 3030(aromatic), 2960, 2930(aliphatic), 2680–2460(NH^+), 1480, 1460, 1440, 970, 770, 760, 750, 740 cm^{-1}. ^1H NMR ($CDCl_3$)δ: 2.05(s, 3H, Ar–CH_3), 2.66(s, 6H, $N(CH_3)_2$), 3.1–3.4(m, 4H, CH_2–CH_2), 7.1–7.4(m, 7H, aromatic), 7.8(1H, aromatic) ppm. ^{13}C NMR ($CDCl_3$)δ: 19.7(q, Ar–CH_3), 23.7(t, CH_2), 42.7(q, $N(CH_3)_2$), 57.7(t, CH_2–N), 122.3(d), 123.0(d), 124.6(d), 124.8(d), 126.2(d), 128.7(d), 130.1(d), 130.7(d), 133.4(s), 133.7(s), 135.8(s), 137.4(s), 138.4(s), 139.7(s) ppm. MS m/z (% rel. int.): 295(0.2 M), 221(4.1), 202(0.9), 59(3.8), 58(100).

Results and Discussion

The photodecomposition of dothiepin in aqueous solutions was carried out in clear glass ampoules, which allow the transmission of wavelengths longer than 300 nm, and in the presence of air. The solutions slowly turned yellow on exposure to light. The nature of the photodecomposition products depended on the radiation source. The similar sensitivity to the radiation source was observed in a previous study on doxepin, an oxygen analogue of dothiepin (Tammilehto et al., 1982).

TLC experiments on dothiepin solutions exposed to artificial radiation revealed one main degradation product, and 2 to 3 minor products present in small quantities. One of the trace compounds was identified as dothiepin sulphoxide by comparing its TLC behaviour with the synthesized reference substance.

The main degradation product, compound III, was easily isolated as its hydrochloride salt by extracting the irradiated dothiepin solutions with chloroform. The compound failed to crystallize as a free base. The results of the elemental analysis and the mass spectrometric data showed compound III to possess the same empirical formula as the parent compound. The IR spectrum differed from that of dothiepin only in the absorption intensities in the fingerprint region. In the mass spectrum the base peak appeared at m/z 58 and was due to the fragment $CH_2 = \overset{+}{N}(CH_3)_2$. This fragmentation process is usual for tricyclic antidepressants having a dimethylaminopropyl sidechain (Biggs et al., 1976). The molecular ion, corresponding to the formula $C_{19}H_{21}NS$, was observed with very poor intensity at m/z 295. Valuable information for the structure elucidation of III was obtained by comparing the NMR spectra of III and dothiepin. The ^1H NMR spectrum of dothiepin shows two characteristic features: a triplet of the methine proton at δ 5.86 ppm and two doublets of the non-equivalent protons of the CH_2 group in the 7-ring at δ 3.40 and 4.82 ppm. Both resonances were lacking in the spectrum of

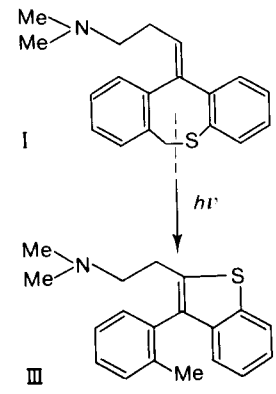

Fig. 1. Structures of dothiepin (I) and photodegradation product (III).

the photodegradation product, indicating the loss of these groups. In contrast, a three-proton singlet at δ 2.05 ppm suggested compound III to have an extra methyl group, possibly formed by opening of the 7-ring (Fig. 1). The ^{13}C NMR spectrum of III confirmed the loss of the CH_2 group of dothiepin at δ 33.2 ppm and the presence of a new methyl carbon at δ 19.7 ppm. According to the off resonance spectrum, compound III contained 6 quaternary carbons, in contrast to the 5 of dothiepin. An explanation could be the formation of a benzothiophene structure (Fig. 1). In the GC analysis compound III gave only one peak, indicating that it does not form geometric isomers. All data obtained pointed to the conclusion that compound III was 2-(2-dimethylaminoethyl)-3-(2-methylphenyl)-benzo[b]thiophene.

In dothiepin solutions exposed to daylight, 5-6 degradation products were detected on TLC plates. None of them was identical with compound III. Two degradation products were isolated by preparative TLC where plates were developed twice with the solvent system toluene-chloroform-1-propanamine (10 : 2 : 1). One of them was identified as dothiepin sulphoxide. The IR spectrum of the other substance was similar to that of dothiepin sulphoxide with the characteristic strong absorption of a sulphoxide group at 1040 cm^{-1} but the amount isolated was insufficient for detailed structure elucidation. On the basis of their TLC behaviour, the substances could be geometric isomers; however, the totally different GC retention times (SE-30 column: 6.26 and 10.60 min) refute this suggestion. Dothiepin sulphone was detected in trace quantities in solutions exposed to daylight for several weeks. Two other degradation products with R_f values higher than that of dothiepin may have lost part of their basic side-chain. This reaction forms the main decomposition mechanism of doxepin under the influence of daylight (Tammilehto and Lehtonen, 1982).

The photodecomposition rate of dothiepin in aqueous solutions irradiated by Hg lamp was followed by glass capillary GC. The geometric isomers of dothiepin and the main decomposition product were well separated on non-polar (OV-101) columns (Fig. 2) as well as on polar (FFAP)

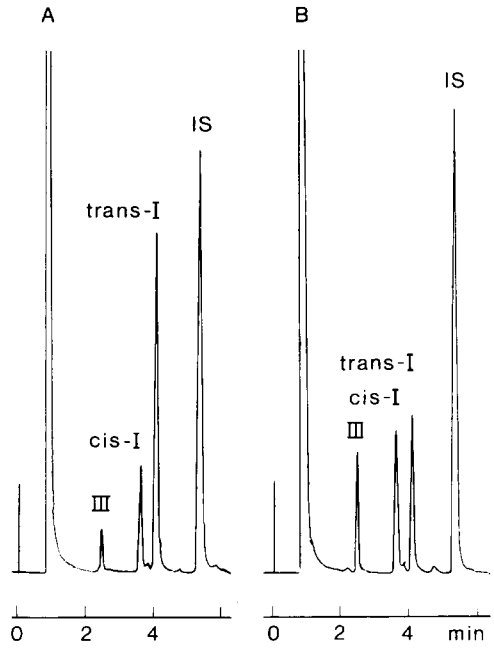

Fig. 2. Chromatograms of partly degraded 0.5% dothiepin hydrochloride solutions at pH 5.6. A, irradiated for 20 min; B, irradiated for 60 min. Radiation source: mercury lamp.

columns. When OV-101 was used as the liquid phase, the retention times were 2.44, 3.64 and 4.13 min for compound III and *cis* and *trans* dothiepin, respectively. The calibration graph showed good linearity in the concentration range studied ($r = 0.999$), and the mean recovery of dothiepin from its 0.5% aqueous solutions was 99.5% (RSD $= \pm 1.56\%$, $n = 12$).

The decrease of the total dothiepin content followed apparent first-order kinetics, the reaction rate depending strongly on the initial drug concentration (Fig. 3). The rate constants were determined from the slopes of the straight lines obtained by plotting the logarithm of the residual dothiepin concentration against time. The half-lives were calculated from the equation $t_{1/2} = 0.693/k$ (Table 2). The pH of the solution seemed to have no marked effect on the degradation, the reaction being only slightly faster in hydrochloric acid than in acetate buffer at pH 5.6. The proportion of the *cis* isomer increased steadily from the initial value of 2% to about 45% in 0.5% dothiepin hydrochloride solution during 60 min of irradia-

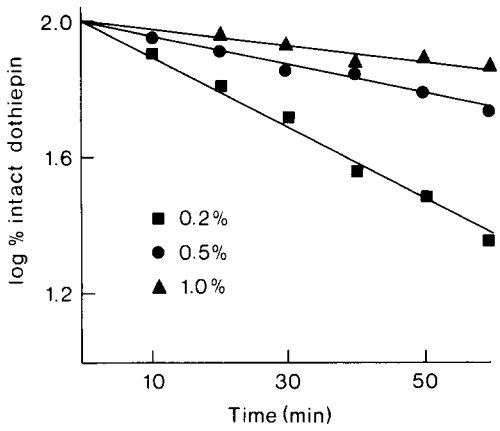

Fig. 3. Effect of drug concentration on the photodegradation of dothiepin hydrochloride at pH 5.6. Radiation source: mercury lamp.

tion. The isomerization and decomposition were negligible after 66 days when the solutions were kept in the dark.

Comparison of the photochemical stability of the two structurally closely related compounds dothiepin and doxepin shows that dothiepin degrades slower (Table 2). The degradation pattern of the two substances is also completely different. Irradiation by the mercury lamp causes rearrangement of the dothiepin molecule to a benzothiophene derivative. The decomposition products of doxepin subjected to artificial radiation have not yet been identified, but on the basis of the TLC experiments the formation of compounds similar to III seems unlikely. Under the influence of daylight, the sulphur atom in the 7-ring of dothiepin is oxidized, and the main degradation products are sulphoxides. Under the same experimental conditions, the side-chain of doxepin is partly or totally split off, and the molecule is oxidized to an aldehyde or ketone (Tammilehto and Lehtonen, 1982).

TABLE 2

Half-lives of dothiepin and doxepin (Tammilehto et al., 1982) in aqueous solutions of different concentrations

Concentration (mg/ml)	Solvent	$t_{1/2}$ (min)	
		Dothiepin	Doxepin
2	Hydrochloric acid 0.1 M	23	–
2	Acetate buffer pH 5.6	28	10
5	Hydrochloric acid 0.1 M	57	29
5	Acetate buffer pH 5.6	68	31
10	Hydrochloric acid 0.1 M	128	–
10	Acetate buffer pH 5.6	132	67

Radiation source: mercury lamp.

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