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Structure elucidation of protriptyline photoirradiation products

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Three photoirradiation products of the tricyclic antidepressant protriptyline have been isolated by preparative tlc and identified as the 10,11-epoxide, 10-hydroxy and 10,11-dihydrodiol derivatives of protriptyline by tlc, uv and mass spectroscopy.

Protriptyline hydrochloride, a tricyclic antidepressant, is reported to cause skin photosensitization in man (Magnus 1976). The mechanism of the cutaneous photosensitivity has not been determined, however protriptyline photosensitises red blood cell lysis (Kochevar & Lamola 1979) indicating that cell membranes may be the site of light-induced damage.

Gasparro & Kochevar (1982) have investigated the photochemical behaviour of protriptyline in the presence and absence of oxygen. They found that in the presence of oxygen, protriptyline solution became yellow and turbid, and hplc analysis showed a complex mixture of photoproducts. In the absence of oxygen however the solution remained clear and hplc analysis showed the presence of two main photoproducts which were not present to any great extent in the presence of oxygen and were responsible for the observed red blood cell lysis. One of these products was identified as the cyclobutyl dimer of protriptyline on uv and ms evidence and the fact that the parent hydrocarbon, dibenzocycloheptatriene, readily dimerises on photoirradiation in the absence of oxygen (Kopecky & Shields 1971). The other main photoproduct was not characterized but was thought not to be a dimer.

The metabolism of protriptyline has been investigated in man, pig and dog (Sisenwine et al 1970) and in rat (Rovei et al 1976). Both groups of workers proposed that the route of metabolism of protriptyline in all these animals was hydroxylation to the 10,11-dihydroprotriptyline diol, via protriptyline, 10,11-epoxide in addition to 10-hydroxylation (Fig. 1). In view of the known toxic effects of such epoxides, e.g. imipramine epoxide (Kappus & Remmer 1975), it was decided to investigate the photodecomposition of protriptyline in aqueous solution in the presence of oxygen to determine if similar epoxides were formed on photoirradiation, which could be responsible for the observed phototoxicity.

Materials and methods

Protriptyline hydrochloride (*N*-methyl 5H-dibenzo (a,d) cycloheptene-5 propylamine, hydrochloride) was generously donated by Merck, Sharp and Dohm Research Laboratories.

Photolysis was carried out using a Hanovia IL medium-pressure mercury vapour arc tube photochemical reactor fitted with a quartz sleeve. In a typical experiment 100 ml of protriptyline hydrochloride (1 mg ml^{-1}) was photoirradiated for 16 h. The resulting solution was basified with NaOH and extracted with Et_2O. The photoirradiation products were separated by preparative tlc of the ethereal extract on silica gel plates (20 × 20 cm) using toluene–ethylacetate–ethanol (95%)-diethylamine (20:20:4:1) as developing solvent. The different bands were visualized by viewing under an uv lamp (254 nm), then scraped off the plates and eluted from the silica with methanol. The methanolic solutions were evaporated to dryness in a stream of nitrogen and the different fractions analysed by tlc, and uv and chemical ionization mass spectroscopy, uv spectra were determined by means of a Cecil CE 505 uv spectrophotometer and chemical ionization mass spectra by means of a Kratos ms 25 mass spectrometer fitted with chemical ionization probe. The ionizing reagent was ammonia.

Authentic samples of the three proposed photodecomposition products were prepared by chemical synthesis.

Protriptyline 10,11-epoxide. Protriptyline 10,11-epoxide was synthesized by peroxidation of protriptyline using *m*-chloroperbenzoic acid (Aldrich Chemical Co. Ltd.)

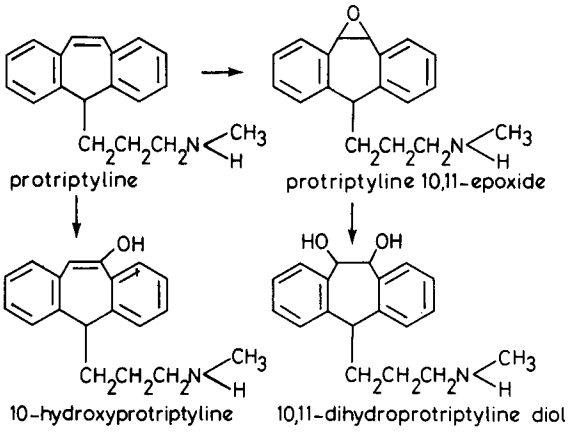

FIG. 1. Metabolic pathways of protriptyline (Rovei et al 1976).

* Correspondence.

Table 1. Tlc and uv spectral data for protriptyline and its photoirradiation products.

Compound	R_F system 1	2	3	uv λ_{max} (nm)
Protriptyline	0·33	0·47	0·7	292
Protriptyline 10,11-epoxide	0·82	0·36	0·6	285
10-Hydroxyprotriptyline	0·18	0·07	0·43	285
10,11-dihydroprotriptyline diol	0·05	0·23	0·58	255
Photoirradiation band 1	0·82	0·36	0·6	285
Photoirradiation band 2	0·18	0·07	0·43	285
Photoirradiation band 3	0·05	0·23	0·58	255

Solvent system 1. Toluene–ethylacetate–ethanol (95%)–diethylamine (20:20:4:1).
Solvent system 2. Hexane–diethylamine (1:1).
Solvent system 3. Ethylacetate–ethanol-diethylamine–H_2O (6:6:6:1).

as oxidizing agent. Protriptyline free base (100 mg) was added to a solution of *m*-chloroperbenzoic acid (100 mg) in dichloromethane (10 ml). The solution was maintained at 25 °C with stirring for 2 h. The precipitated benzoic acid was removed by extraction with 10% NaOH solution. The dichloromethane solution was washed with water, dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure to yield a colourless oil (50 mg) which was shown to be chromatographically pure by tlc in several solvent systems and identified as protriptyline 10,11-epoxide by chemical ionization mass spectroscopy.

10,11-Hydroxyprotriptyline and 10,11-dihydroprotriptyline diol. 10-hydroxyprotriptyline and 10,11-dihydroprotriptyline diol were synthesized by performic acid oxidation of protriptyline (Vogel 1956). Protriptyline free base (1 g) was added slowly to a mixture of 90% formic acid (7 ml) and 30% hydrogen peroxide (2 ml). The initial vigorous reaction was cooled in an ice bath and the reaction mixture maintained at 40 °C for 1 h and then left to stand at room temperature (20 °C) for 16 h. The solution was made alkaline with cold NaOH and extracted with ethyl acetate. The ethyl acetate extract was found to give two spots (R_F 0·18 and 0·05) on silica gel tlc, developing solvent, toluene–ethyl acetate–ethanol (95%)–diethylamine (20:20:4:1). The two compounds were separated by preparative tlc using the same solvent system to yield white crystalline solids which were identified by chemical ionization mass spectroscopy as 10-hydroxyprotriptyline and 10,11-dihydroprotriptyline diol respectively.

Results and discussion

On photoirradiation for 16 h in the presence of oxygen, protriptyline hydrochloride yielded a pale yellow solution which on tlc examination was found to contain three bands. The R_F values and uv spectral characterisitcs of the separated bands compared to the R_F values and spectral characteristics of synthetic reference compounds indicated that the composition of the bands was band 1, protriptyline 10,11-epoxide, band 2, 10-hydroxyprotriptyline and band 3, 10,11-dihydroprotriptyline diol (Table 1).

Table 2. Mass spectral analysis of protriptyline photoirradiation products.

Compound	Peaks *m/z* (relative abundance)
Protriptyline	264 (100%), 191 (9·9%), 70 (25·2%), 44 (24·7%)
Protriptyline 10,11-epoxide	280 (19%), 207 (3%), 178 (4·2%), 70 (17%), 44 (100%)
10-Hydroxyprotriptyline	280 (4·3%), 279 (5·0%), 207 (5·7%), 70 (29·5%), 44 (100%)
10,11-Dihydroprotriptyline diol	298 (4·4%), 281 (21·3%), 250 (45·9%), 207 (22·8%), 179 (39·7%), 70 (81·2%), 44 (100%)
Photoirradiation band 1	280 (17·3%), 207 (3·7%), 178 (3·7%), 70 (15·6%), 44 (100%)
Photoirradiation band 2	280 (3·9%), 279 (4·7%), 207 (5·4%), 70 (32·1%), 44 (100%)
Photoirradiation band 3	298 (6·5%), 281 (60·1%), 250 (8·7%), 207 (9·0%), 179 (19·6%), 70 (77·6%), 44 (100%)

These findings were confirmed by a comparison of the chemical ionization mass spectra of the isolated bands with those for the synthetic reference compounds Table 2). Band 1 gave a M + 1 peak at *m/z* 280 which suggested the introduction of an oxygen atom into the protriptyline molecule and fragmentation peaks at *m/z* 207 indicating the loss of the amino alkyl side chain (cf. the fragmentation of protriptyline) and at *m/z* 178 indicating the loss of CHO and suggesting the presence of an epoxide linkage.

Band 2 gave a peak at *m/z* 280 similarly indicating the presence of an oxygen atom and a fragmentation peak at *m/z* 207 indicating the loss of the aminoalkyl side chain.

Band 3 gave a M + 1 peak at 298 indicating the introduction of two hydroxyl groups into the protriptyline molecule and fragmentation peaks at *m/z* 281 (loss of OH) and at *m/z* 179 arising from the ion at *m/z* 207 by loss of ethylene.

All spectra gave peaks at *m/z* 70 and *m/z* 44 due to fragmentation of the aminoalkyl side chain and were comparable to the respective electron impact mass spectra published by Rovei et al (1976).

Thus, on photoirradiation, protriptyline is oxidized to a protriptyline diol via an active epoxide. A similar epoxide is formed on photoirradiation of chordiazepoxide and has been shown to be responsible for the phototoxic properties of that compound (Cornelissen 1981). It would seem probable therefore that the reported phototoxicity of protriptyline could be caused by an irreversible reaction between protriptyline 10,11-epoxide and cellular macromolecules, in a similar way to the irreversable binding of imipramine epoxide to albumins reported by Kappus & Remmer (1975).

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Biochemical effects of minaprine on striatal dopaminergic neurons in rats

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The biochemical effects of minaprine, a new psychotropic drug, were investigated on striatal dopaminergic neurons in the rat. Minaprine did not displace [^{3}H]spiperone in-vitro binding from striatal membranes but had clear effects on dopamine (DA) metabolites. Homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were significantly decreased in a dose-dependent manner after intraperitoneal administration of minaprine 30 min before killing. In rats injected with minaprine 15 mg kg^{-1} i.p. at different intervals, the decrease in striatal HVA and DOPAC was time-dependent and a concomitant rise in 3-methoxytryramine (3-MT) concentrations was observed. The maximum of these effects was reached 30 min after minaprine. When administered 5 min after a monoamineoxidase (MAO) inhibitor (pargyline, 100 mg kg^{-1} i.p.) and 30 min before killing, minaprine did not affect pargyline-induced changes in HVA, DOPAC and 3-MT levels. This together with other data suggests that minaprine affects DA metabolism by acting, at least partially, at presynaptic level through in-vivo inhibition of MAO activity.

Minaprine (CM30038, morpholinoethylamino-3-methyl-4-phenyl-6-pyridazine) is a novel psychotropic drug synthesized by Wermuth & Exinger (1972); it has convulsant activity and blocks glycine receptors. Biochemical studies have shown that minaprine raises 5-hydroxytryptamine (5-HT) concentration in striatum, hypothalamus, parietal cortex and raphe region. (P. Mandel, personal communication) and induces a marked increase of acetylcholine concentration and a reduction of the dopamine (DA) turnover in the striatum (Garattini 1981).

The mechanism of action on DA metabolism changes induced by minaprine in rat striatum was investigated by measuring its main catabolites: homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) concentrations mainly give an index of the intraneuronal metabolism and 3-methoxytyramine (3-MT), produced in the synaptic cleft, has been proposed as a better indicator of DA release (Carlsson & Lindqvist 1963; Kehr 1976; Di Giulio et al 1978).

* Correspondence.

Methods

Male CD-COBS rats (Charles River, Italy) 225–250 g, housed in standard conditions (60% relative humidity, 22 °C, 12-h light-dark cycles) with free access to water and food were injected intraperitoneally with minaprine (kindly supplied by Sanofi, Montpellier, France), at different doses, or pargyline HCl (Aldrich Chem. Comp., USA) 100 mg kg^{-1}, or both. The drugs were dissolved in water and injected in a volume of 2 ml kg^{-1}. Rats were killed at different intervals by microwave irradiation (1·3 W at 2·45 GHz for 4·25 s) to rapidly inactivate catechol-*O*-methyltransferase (COMT). Striata were rapidly dissected, frozen on dry ice and kept at −80 °C until biochemical assay.

For the simultaneous assay of HVA, DOPAC, 3-MT and DA the striata of each animal were homogenized by sonication (Branson Sonifier B 15) in 300 µl of 0·4 M perchloric acid and centrifuged at 10 000 rev min^{-1} for 10 min. The clear supernatant was divided into two parts: 50 µl was used for HVA and DOPAC assay according to a method previously described by Ponzio & Jonsson (1978) and 250 µl was adjusted to pH 7·5–7·6 to separate DA from 3-MT. At this pH, DA was adsorbed onto alumina and 3-MT remained in the supernatant from which it was extracted according to Ponzio et al (1981a). DA was eluted from alumina according to Keller et al (1976). Liquid chromatography with electrochemical detection (LCEC) (Bioanalytical Systems Inc., West Lafayette, Ind.) was used for all biochemical determinations. A glass column 500 mm long, 2 mm i.d., packed with a cation exchange resin (Vydac CX, P310. The Separation Group Hesperia, CA) was used for DA determination. Samples were eluted with citrate-acetate buffer pH 5·2, 0·33 M; detector potential +0·75 V.

HVA and DOPAC were separated in a glass column 750 mm long, 2 mm i.d., packed with anionic exchange resin (Zipax SAX Du Pont) using citrate-acetate buffer pH 7·4, 0·04 M as eluant; detector electrode potential: +0·75 V.

3-MT was separated in a glass column 300 mm long, 2 mm i.d., packed with cation exchange resin Vydac P