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Photoirradiation products of cyproheptadine and carbamazepine

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Cyclobutyl dimer and 10,11-epoxide photoirradiation products of cyproheptadine and carbamazepine have been isolated by preparative tlc and identified by tlc, uv, pmr and mass spectroscopy.

The tricyclic antidepressant drug protriptyline has recently been shown to produce potentially toxic photoproducts such as the cyclobutyl dimer (Gasparro & Kochevar 1982) and the 10,11-epoxide (Jones & Sharples 1984) on photoirradiation. This communication reports the structure elucidation of the photoirradiation products of two structurally similar drugs, cyproheptidine and carbamazepine.

Materials and methods

Cyproheptadine hydrochloride and carbamazepine were generously donated by Merck, Sharp and Dohm research laboratories and CIBA-GEIGY Pharmaceuticals respectively. Photolysis, separation and identification of photoirradiation products were carried out as previously reported for protriptyline (Jones & Sharples 1984).

Authentic samples of cyproheptadine 10,11-epoxide and carbamazepine 10,11-epoxide were synthesised by peroxidation of cyproheptadine and carbamazepine respectively using *m*-chloroperbenzoic acid as oxidizing agent (Jones & Sharples 1984).

Results and discussion

(i) *Photoirradiation of cyproheptadine hydrochloride.* An aqueous solution of 100 ml cyproheptadine hydrochloride (1 mg ml^{-1}) was photoirradiated for 16 h in the presence of oxygen. After 4 h a white crystalline precipitate (50 mg) was produced. This was filtered off, and dried under vacuum. Photoirradiation of the solution was continued. After 16 h a pale yellow solution was obtained which was made alkaline with NaOH and extracted with ether. Tlc examination of the ethereal extract showed the presence of two bands. The R_F values and uv spectral characteristics of these separated bands compared with those of reference compounds indicated that the compositions of these bands was: band 1, cyproheptadine 10,11-epoxide and band 2, unchanged cyproheptadine (Table 1). These findings were confirmed by a comparison of the chemical ionisation mass spectra of the isolated bands with those for the appropriate reference compounds (Table 2). The white crystalline precipitate was identified as the cyclobutyl dimer of cyproheptadine on the following evidence: melting point (capillary) 295 °C (not previously reported), uv λ_{max} (methanol) 220 nm, chemical ionization ms $M^+ + 2$ peak *m/z* 576, (11·4%) fragmentation peaks at *m/z* 288, (100) 274, (6·7) 215, (3·3) pmr spectrum ($CDCl_3$) 7·2δ multiplet, (16H), aromatic protons, 4.0δ singlet (4H), cyclobutyl protons, 2·3–2·7δ multiplet (8H), piperidine ring protons, 1·3δ singlet (6H), *N*-methyl protons. The pmr spectrum was comparable to that of the photodimer of 5H-dibenzo[a,d]cyclohepten-5-one (Kopecky & Shields 1971).

(ii) *Photoirradiation of carbamazepine.* An aqueous solution of 100 ml carbamazepine (1 mg ml^{-1}) was photoirradiated for 16 h in the presence of oxygen.

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Table 1. Tlc and uv spectral data for cyproheptadine, carbamazepine and their photoirradiation products.

Compound	R_F System 1	System 2	System 3	System 4	uv λ_{max} nm
Cyproheptadine	0·25	0·72	0·40	—	223, 285
Cyproheptadine 10,11 epoxide	0·04	0·43	0·19	—	222, 284
Photo product I	0·05	0·42	0·19	—	220, 285
Photo product II	0·23	0·73	0·40	—	223, 285
Carbamazepine	—	—	—	0·35	215, 285
Carbamazepine 10,11-epoxide	—	—	—	0·21	210
Photo product I	—	—	—	0·21	210
Photo product II	—	—	—	0·33	215, 285

Solvent system 1, toluene–ethylacetate–ethanol (95%)-diethylamine (20 : 20 : 4 : 1).
Solvent system 2, n-hexane–diethylamine (1 : 1).
Solvent system 3, carbontetrachloride–methanol–diethylamine (85 : 10 : 5).
Sovlent system 4, toluene–glacial acetic acid (80 : 20).

Table 2. Mass spectral analysis of cyproheptadine and carbamazepine photoirradiation products.

Compound	Peaks *m/z* (relative abundance)
Cyproheptadine	288 (100%), 273 (26%), 215 (64%), 96 (41%), 70 (19%)
Cyproheptadine 10,11-epoxide	304 (32%), 288 (47%), 274 (28%), 202 (100%) 96 (5·0%)
Photo product I	304 (53%), 288 (100%), 274 (26%), 202 (22%), 96 (16%)
Photoproduct II	288 (100%), 273 (13%), 215 (57%), 96 (34%), 70 (11%)
Carbamazepine	237 (33%), 194 (100%)
Carbamazepine 10,11-epoxide	253 (29%), 237 (100%), 224 (31%), 181 (25%)
Photo product I	253 (3%), 237 (100%), 224 (15%), 181 (8%)
Photoproduct II	237 (100%), 194 (64%)

After 2 h a white crystalline precipitate (30 mg) was produced. This was filtered off and dried under vacuum. Photo-irradiation of the solution was continued. After 16 h a colourless solution was obtained which was extracted with chloroform. Tlc examination of the chloroform extract showed the presence of two bands. The R_F values and uv spectral characteristics of these separated bands compared with those of reference compounds indicated that the composition of these bands was: band 1, carbamazepine 10,11-epoxide and band 2, unchanged carbamazepine (Table 1). These findings were confirmed by a comparison of the chemical ionisation mass spectra of the isolated bands with those for the appropriate reference compounds (Table 2). The white crystalline precipitate was identified as the cyclobutyl dimer of carbamazepine on the following evidence: Melting point (capillary), 370 °C, Kricka et al (1974) give 367–370 °C, uv λ_{max} (methanol) 208 nm, chemical ionization ms $M^+ + 1$ peak *m/z* 473 (29%), and fragmentation peaks at *m/z* 237 (100) and *m/z* 194 (54), pmr spectrum (CF_3COOD) 7·3 δ multiplet, (16H), aromatic protons, 4·1 δ singlet, (4H), cyclobutyl protons, 0·9 δ singlet (4H) exchangeable with D_2O, NH_2 protons.

Thus, it can be concluded that the major photoirradiation product of both cyproheptadine and carbamazepine under these conditions is the cyclobutyl dimer. Also produced are small amounts of the respective 10,11-epoxides.

In view of the ease with which protriptyline, cyproheptadine and carbamazepine form these cyclobutyl dimers one might speculate that the mechanism of photoinduced toxicity with these compounds might be by formation of cyclobutyl adducts with the pyrimidine bases of DNA in an analogous way to the furanocoumarins (Murajo et al 1967).

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On the usefulness of ultrafiltration in drug-protein binding studies

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Severe non-specific adsorption of verapamil, nifedipine, prazosin and nadolol was observed during ultrafiltration of the drug solutions through the Centriflo CF 50A, YMT, YMB and Visking membranes. The results question the adequacy of the ultrafiltration procedure for the protein binding assay of the tested drugs.

Protein binding of drugs in plasma and its role in pharmacokinetics and the dynamics of the pharmacological response have attracted attention in recent years (McNamara et al 1979; Øie et al 1980; Levy 1980). An essential methodological aspect of these studies is choice of the assay procedure, device and materials. Equilibrium dialysis is most frequently used for these purposes, however it is time-consuming and leads to sample dilution. Attempts have been made to achieve more rapid separation of free and protein-bound drugs using various modifications of ultrafiltration. During our pharmacokinetic studies of cardiovascular drugs, we turned to ultrafiltration for the determination of the protein binding of verapamil, nifedipine, prazosin and nadolol in serum of patients treated with these drugs. In the preliminary experiments reported here we have tested the binding of the drugs to the membrane filters routinely used for utlrafiltration. The results question the adequacy of the ultrafiltration procedure for the protein binding assay.

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Materials and methods

Membranes of four types were tested by means of centrifugal ultrafiltration: Centriflo CF 50A conical membrane filters, YMT and YMB membranes for the MPS 1 micro partition system (Amicon, USA) and Visking dialysis tubing (type 8/32, Serva, FRG). In the last case, the method of Heckman & van Ginneken (1982) was used. Drug solutions were prepared in