Fluorescence Characteristics of Azaperone and of an Azaperone Mononitrogen Oxide

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Abstract □ The structure elucidation of the compound isolated after peroxide treatment of azaperone is described. A mononitrogen oxide was formed at the piperazine N_1 atom after reaction with excess hydrogen peroxide. The fluorescence characteristics of the derivative were examined and compared with the native fluorescence capacities of the azaperone base; both were identical, depending on the solvent nature. The phenomenon is explained by the fact that the fluorescent properties of the azaperone molecule are principally produced by its *ortho*-nitrogen-substituted pyridine nucleus.

Keyphrases □ Azaperone and oxide—fluorescence characteristics, effect of pH □ Fluorescence characteristics—azaperone and oxide, effect of pH □ Tranquilizers—azaperone and oxide, fluorescence characteristics, effect of pH

Azaperone (I), 1-(4-fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-1-butanone, is a butyrophenone neuroleptic. Butyrophenones can be considered as 4-aminobutyric acid derivatives, a prototype of which, haloperidol, is frequently used as an antipsychotic and antiemetic agent. Pharmacologically, the butyrophenones resemble the neuroleptic phenothiazines (1–3). Azaperone is commonly used in veterinary medicine. It occurs as an almost white to slightly yellowish amorphous or crystalline powder, is nearly insoluble in water, and is soluble in dilute acids and organic solvents.

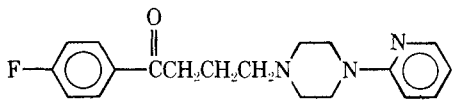
During the spectrofluorometric investigation of some therapeutically used butyrophenones (4), the remarkable fluorescence characteristics of the azaperone molecule were noted and shown to be caused by the heterocyclic substituent of the γ-aminobutyric acid chain.

The purpose of this investigation was to determine whether nitrogen oxidation of the azaperone molecule would affect its native fluorescence characteristics. These experiments were performed simultaneously with spectrofluorometric analyses of some mono- and diaminopyridine isomers before and after peroxide treatment because of the general shortage of literature references concerning fluorometric data of aminopyridines, which frequently occur as molecular fractions in various drugs.

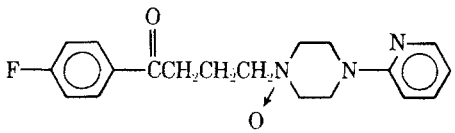
The present paper reports the preparation and structure elucidation of the compound resulting from the reaction of azaperone with concentrated hydrogen peroxide.

EXPERIMENTAL[1]

Apparatus—A recording spectrophotofluorometer was used for recording excitation and emission spectra and for measuring fluorescence intensities. Spectra were not corrected for variations in lamp intensity or photomultiplier sensitivity.



I



II

Chemicals—All chemicals[2] were analytical grade.

Preparation of Solutions for Fluorescence Measurement—All solutions measured fluorometrically contained 0.5–10 μg of azaperone or derivative/ml. All glassware should be free of fluorescent contaminants and, therefore, frequently rinsed with distilled water; quartz sample cells are preferably cleaned with nitric acid followed by distilled water.

Preparation of Azaperone Peroxide Derivative (II)—Azaperone (9.16 mmoles, 3 g) was dissolved in methanol (100 ml); hydrogen peroxide (490 mmoles, 50 ml of 30% w/w) was added, and the reaction mixture was placed in a boiling water bath for 1 hr with frequent stirring. The solution was allowed to cool at room temperature and was subsequently cooled in ice. If no crystallization occurred, further evaporation was required.

A white precipitate appeared along with a yellow, oily phase. Scratching the sides of the glass vessel with a glass rod finally provided a homogeneous white crystalline product. After filtration, the residue was recrystallized a few times from 50% (v/v) methanol–water and finally dried at 30–40°. An 80% yield was obtained.

A stoichiometrically similar hydrogen peroxide treatment of the two molecular fractions of azaperone was performed. 1-(4-Fluorophenyl)-1-butanone[3] apparently was not attacked in these circumstances. 1-(2-Pyridyl)piperazine[3], however, was converted into a mixture in which at least 10 components could be distinguished by TLC but none of which could be crystallized.

RESULTS

The isolated II appeared as a white crystalline powder, mp 138° (azaperone mp 90°), and was stable on storage at room temperature in the dark. It is very soluble in water and methanol, soluble in chloroform, and less soluble in acetone and ether. The compound can release iodine from an aqueous acid solution of potassium iodide at room temperature.

The UV maxima of II were: (methanol) 244 (ε 25,400)[4] and 298 (ε 2500) nm and (chloroform) 247 (ε 30,400) and 298 (ε 3800) nm. The UV maxima of azaperone were: (methanol) 247 (ε 28,400) and 304 (ε 4100) nm and (chloroform) 248 (22,900) and 305 (ε 2100) nm.

Excitation and fluorescence spectra were obtained from azaperone, II, and 1-(2-pyridyl)piperazine dissolved in various solvents in a concentration of 0.5 μg/ml. In each case, identical results were obtained for the three compounds. For example, in 0.1 *N* sulfuric acid, λ_{exc} = 315 nm and λ_{em} = 405 nm. In 20% (v/v) methanol–water, λ_{exc} = (245)[5] 300 nm and λ_{em} = 375 nm. In 0.1 *N* sulfuric acid in 20% (v/v) methanol–water, λ_{exc} = (245) 315 nm and λ_{em} = 405 nm. Variation of the acid strength up to 20% (w/v) yielded identical spectra. When pure organic solvents (*e.g.*, methanol, ethanol, 2-propanol, 2-methoxyethanol, ethyl acetate, and

[1] The instruments used were: an Aminco-Bowman spectrofluorometer, Catalog No. 4-8203 DE, American Instrument Co., Silver Spring, Md.; a Hanovia 150-w xenon arc lamp; two grating monochromators; 1 × 1-cm quartz cells; photomultiplier R 446 S with high voltage of 0.7 kv and slits varying between 0.5 and 2 mm; a Zeiss DMR 21 spectrophotometer; a Beckman IR 7 spectrometer; an L.K.B. 9000 S mass spectrometer; and a Varian HA-100 NMR spectrometer, Varian Associates, with a permanent frequency of 100 MHz. Melting points were taken on a Büchi apparatus. TLC plates were visualized with a Camag TL 900 Universal UV lamp at 254 and 350 nm.

[2] E. Merck, Darmstadt, West Germany.

[3] Aldrich Chemicals, Beerse, Belgium.

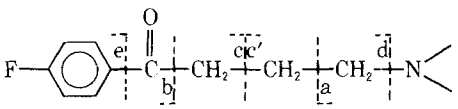
[4] Approximate molar absorptivities were calculated using 327.4 as the molecular weight of azaperone and 343.4 for II, as will be shown from the mass spectral data.

[5] Numbers in parentheses refer to a secondary excitation value of much less intensity.

chloroform) were used, similar results were obtained, with the maxima depending on the polarity of the solvent. The detection limit of both compounds determined fluorometrically in 0.1 *N* sulfuric acid was 1×10^{-2} μg/ml.

IR absorption spectra were taken by dissolving 1 mg in methanol and mixing it with 300 mg of potassium bromide (spectroscopic grade) until complete evaporation occurred. The mixture was then compressed into a tablet. Azaperone absorption bands were: 3100–3000 (aromatic =CH), 3000–2700 (CH_2), 1680 (aryl ketone C=O), 1593–1503–1475 (aromatic C=C), 1590–1500 (C=N), 1228 (CF), 1200–1100–1000 and 730–650 (CH deformations, pyridine nucleus ring vibrations), 825 (CH out of plane: 1,4-aromatic substituents), and 772 (CH out of plane: 1,2-aromatic substituents, piperazine ring vibrations) cm^{-1}. The Compound II spectrum was similar to the azaperone spectrum with minute changes in the fingerprint region: 1400–1350, 1350–1210, 1200–1100, and 875–725 cm^{-1}; strong absorption at 933 (>N → O nonaromatic) cm^{-1}.

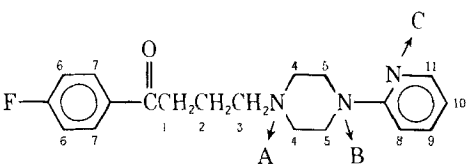
Mass spectral data (70 ev) of azaperone differed from the reported fragmentation paths of butyrophenones (5–9) because of the pyridine nitrogen involved in the fragmentation process (Structure III):



III

m/e (relative intensity) 327 (6) ($M^{+\cdot}$), 309 (4) ($M^{+\cdot} - H_2O$) (m* at 292), 189 (8) (c′, Mc Lafferty), 176 (9) (a, usually base peak in other butyrophenones), 165 (21) (d), 133 (10) ($C_8H_9N_2$, piperazine fragmentation), 123 (30) (b, *p*-fluorobenzoyl ion), 107 (100) (base peak $C_6H_7N_2$), 95 (27) (e, *p*-fluorophenyl ion from *m/e* 123 − CO), and 79 (25)–78 (22) (pyridine fragmentations).

The mass spectrum for II (70 ev) was similar to the azaperone spectrum: *m/e* 343 (3) ($M^{+\cdot}$ azaperone + 16), 327 (3) ($M^{+\cdot} - O$), 326 (4) ($M^{+\cdot} - OH$), 325 (12) ($M^{+\cdot} - H_2O$) in order of increasing intensity (10), 309 (3) ($M^{+\cdot} - H_2O$), 165 (12) (d), 162 (57) (pyridylpiperazine$^+$ after loss of oxygen), 133 (24) ($C_8H_9N_2$), 123 (100) (base peak *p*-fluorobenzoyl nucleus), 107 (63) (base peak azaperone), 95 (45) (*p*-fluorophenyl ion), and 79 (17)–78 (23) (pyridine fragment ions).



IV

NMR[6] of azaperone (Structure IV) (deuterochloroform, 1% tetramethylsilane) showed: δ 1.97 (2 × t, 2, $J_{2,1}$ = 7 Hz, $J_{2,3}$ = 7.5 Hz, H-2), 2.44 (t, 2, $J_{3,2}$ = 7.5 Hz, H-3), 2.52 (t, 4, $J_{4,5}$ = 5 Hz, H-4), 2.98 (t, 2, $J_{1,2}$ = 7 Hz, H-1), 3.47 (t, 4, $J_{5,4}$ = 5 Hz, H-5), 6.56 (2 × d, 1, $J_{10,9}$ = 7 Hz, $J_{10,11}$ = 5 Hz, $J_{10,8}$ = 2.3 Hz, H-10), 6.60 (d, 1, $J_{8,9}$ = 7.5 Hz, $J_{8,10}$ = 2.3 Hz, H-8), 7.09 (t, 2, $J_{6,7}$ = 8.5 Hz, $J_{6,F}$ = 8.5 Hz, H-6), 7.47 (2 × d, 1, $J_{9,8}$ = 7.5 Hz, $J_{9,10}$ = 7 Hz, $J_{9,11}$ = 2 Hz, H-9), 7.94 (2 × d, 2, $J_{7,6}$ = 8.5 Hz, $J_{7,F}$ = 5.5 Hz, H-7), and 8.14 (d, 1, $J_{11,10}$ = 5 Hz, $J_{11,9}$ = 2 Hz, H-11) ppm.

NMR of Compound II (methanol-d_4, 1% tetramethylsilane) showed: δ 2.29 (m, 2, H-2), 3.30–3.80 (m, 12, H-1, 3, 4, and 5), 6.68 (2 × d, 1, $J_{10,9}$ = 7 Hz, $J_{10,11}$ = 5 Hz, H-10), 6.72 (d, 1, $J_{8,9}$ = 8.5 Hz, $J_{8,10}$ = 1 Hz, H-8), 7.19 (t, 2, $J_{6,7}$ = 8 Hz, $J_{6,F}$ = 8 Hz, H-6), 7.58 (2 × d, 1, $J_{9,8}$ = 8.5 Hz, $J_{9,10}$ = 7 Hz, $J_{9,11}$ = 2 Hz, H-9), 8.07 (2 × d, 2, $J_{7,6}$ = 8 Hz, $J_{7,F}$ = 5 Hz, H-7), and 8.10 (m, 1, H-11) ppm.

Anal.—Calc. for $C_{19}H_{22}FN_3O_2$: C, 66.46; H, 6.46; F, 5.53; N, 12.24. Found: C, 66.43; H, 6.51; F, 5.55; N, 12.22.

DISCUSSION

Since no data were available concerning the formation of nitrogen oxides of the butyrophenone derivatives, an attempt was made to work out a suitable reaction scheme starting from some general rules (11).

Methanolic solutions of azaperone were treated with aqueous solutions of hydrogen peroxide of different strengths at varying pH values and times of reaction. The described method for converting the azaperone base into a mononitrogen derivative using a large excess of hydrogen peroxide originally produced a mixture containing at least three components; one occurred in relatively large amounts, as checked by TLC. It was this component that was purified in the recrystallization process and that fluoresced blue on chromatographic plates in an acid medium on UV exposure. The other substances did not show this phenomenon.

Some of the TLC systems tested to control the purity and stability of synthesized II are shown in Table I. As the azaperone base, its peroxide derivative showed a weak blue native fluorescence on thin layers that was remarkably intensified on contact with acids. In Solvent System 4, for example, both substances were very distinctly recognizable as blue spots on irradiation at 350 nm. Treatment with ammonia vapors largely inhibited the fluorescense emission.

The UV absorption spectra of azaperone and II only demonstrated small differences, as expected. The peroxide derivative showed a slight hypsochromic shift. Absorption in the 300-nm range was mainly caused by the 1-(2-pyridyl)piperazine fraction in the molecule. Absorption in the 240-nm area was due to this fraction as well as to the 1-(*p*-fluorophenyl)-1-butanone substituent, as was noticed after registration of the spectra of these pure compounds in identical conditions.

The azaperone molecule possessed intense fluorescent properties in various solvents because of the heterocyclic pyridylpiperazine substitution of the γ-aminobutyric acid chain. These properties were strongly influenced by the acidity of the medium. Table II illustrates the qualitative effect of pH on the native fluorescence of azaperone. Between the extreme excitation maxima, there was an area of 20 nm; between the two extreme emission maxima, the difference was 42 nm.

Analogous results were obtained with methanol, 2-propanol, propylene glycol, 2-methoxyethanol, and dimethylformamide. Measurements were made on two volumes of azaperone (50 μg/ml of solvent) mixed with one volume of buffer solution. Care should be taken to avoid possible precipitation in the alkaline medium. The compositions of the buffer solutions were: 0.2 *M* potassium chloride–0.2 *N* hydrochloric acid (pH 1.0 and 2.0), 0.1 *M* potassium biphthalate–0.1 *N* hydrochloric acid (pH 3.0 and 4.0), 0.1 *M* potassium biphthalate–0.1 *N* sodium hydroxide (pH 5.0), 0.1 *M* monobasic potassium phosphate–0.1 *N* sodium hydroxide (pH 6.0, 7.0, and 8.0), 0.1 *M* boric acid in 0.1 *M* potassium chloride–0.1 *N* sodium hydroxide (pH 9.0 and 10.0), and 0.1 *M* glycine in 0.1 *M* sodium chloride–0.1 *N* sodium hydroxide (pH 11.0 and 12.0).

Azaperone fluoresced most intensely in an acid medium with an optimum at pH 2. These fluorescent properties at low pH values as well as in pure organic solvents or in aqueous solutions were worked out quantitatively (4). Figure 1 illustrates the excitation and emission spectra of azaperone and II in an acid medium. Both compounds showed identical fluorescent properties, qualitatively and quantitatively. Pure 1-(2-pyridyl)piperazine also yielded analogous spectra. 1-(4-Fluorophenyl)-1-

Table I—TLC R_f Values[a] of I and II

Solvent System	I	II
1. Chloroform	0.02	0.00
2. Chloroform–methanol (90:10 v/v)	0.69	0.12
3. Methanol–28% (w/w) ammonia (100:1.5 v/v)	0.72	0.58
4. Benzene–methanol–acetic acid (80:15:5 v/v)	0.42	0.47
5. Benzene–acetone–petroleum ether (bp 25–70°) –28% (w/w) ammonia (35:35:35:1 v/v)	0.80	0.00
6. Acetone	0.53	0.00

[a] Five micrograms of each compound was spotted on a silica gel 60 F_{254} 0.25-mm plate (Merck). Visualization at 254 nm.

Table II—Influence of Acidity on Fluorescence Characteristics of Azaperone in Ethanol

pH	λ_{exc}, nm	λ_{em}, nm
1	325	403
2	322	398
3	314	365
4	313	361
5	312	367
6	305	367
7	308	373
8	316	370
9	310	375
10	309	376
11	309	378
12	310	378

[6] In NMR descriptions, s = singlet, d = doublet, 2 × d = doublet of doublets, t = triplet, and m = multiplet.

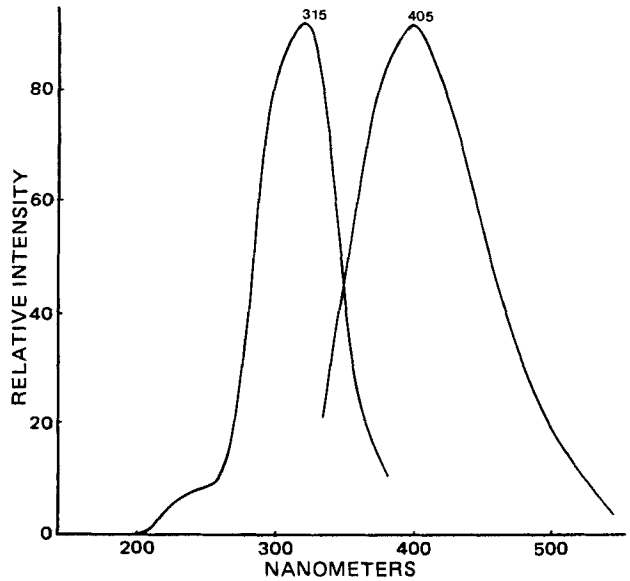


Figure 1—*Activation and emission spectra of azaperone and its peroxide derivatve in 0.1* N *sulfuric acid, 0.5 μg/ml;* λ_{exc} *= 315 nm and* λ_{em} *= 405 nm.*

butanone, dissolved in an organic solvent such as methanol, showed no fluorescent properties at all, as expected.

The NMR spectrometric data show that oxidation apparently occurred at nitrogen atom A of the piperazine ring system. Only this selective oxidation can explain the considerable downfield shift of protons 1, 2, 3, and 4. *N*-Oxidation at nitrogen atom B would merely cause a significant downshift at protons 4 and 5; for nitrogen C, the shift would occur at protons 8, 9, 10, and 11. Integration of the protons of azaperone and II yielded 22 protons for both compounds. This result and the elemental analysis results and mass spectral data suggest that the isolated azaperone peroxide derivative has Structure II.

Compound II possessed identical fluorescent properties as the azaperone molecule in different solvents.

The absence of any influence of *N*-oxidation on the native fluorescence characteristics of the azaperone molecule confirms the localization of the amine oxide formation. The nucleus of the electronic transitions leading to fluorescence emission is situated in the aromatic aminopyridine substituent. Modification of this ring or of the *ortho*-nitrogen substituent certainly would result in an alteration of the fluorescent properties of the molecule by directly influencing the π-electrons of the aromatic system.

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