Degradation of Propildazine in Water

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Abstract □ Propildazine degradation in water at pH 7.4 was studied under aerobic conditions. Three main products were isolated by column chromatography and identified on the basis of IR, UV, and NMR spectroscopy and mass spectrometry and by comparison with synthetic samples. Drug disappearance in water was monitored by UV spectroscopy and was concentration, pH, temperature, and oxygen dependent. Propildazine degradation also was followed in methanol at various pH values.

Keyphrases □ Propildazine—degradation in water, ionization constants, pKa determination, identification of degradation products □ Antihypertensive agents—propildazine, degradation in water, identification of degradation products □ Degradation—propildazine in water and methanolic solutions, determination of products

Propildazine (I) is a new antihypertensive drug. Because of its potent peripheral vasodilatory action (1, 2), it has been subjected to pharmacological screening (3–5) and clinical trials (6–10). Compound I can be stored easily as the dihydrochloride, but its conjugated bases (monohydrochloride and free base) are, like hydralazine (11), unstable. Therefore, good characterization and understanding of the stability of I in solution are needed for pharmacokinetic and preformulation studies.

This study identified the major degradation products resulting from the hydrolysis of I at pH 7.4; in addition, estimates were made of its hydrolysis in aqueous buffers at pH 6.0, 7.4, and 9.0 at 26 and 37°. Isolation of the degradation products was carried out by preliminary column chromatography and subsequent monitoring by TLC. New structures were elucidated by IR, UV, NMR, and mass spectral techniques. For analytical purposes (12), decomposition of I in methanolic solution was also followed.

EXPERIMENTAL

Materials—All chemicals were reagent grade. Distilled water was used for preparing aqueous solutions[1]. Spectrophotometric grade methanol was used for UV determinations. The identity and purity of I were verified by IR[2], UV[3], NMR[4], and mass[5] spectra, melting point (215–218° dec.), elemental analysis, and TLC [silica gel plates[6] buffered at pH 5; eluent of acetone–0.1 *N* HCl (70:10); R_f 0.68].

Determination of Ionization Constants—The pKa values of propildazine were determined at 25°. A 10-ml sample of a 3.7×10^{-3} *M* solution of I in water was titrated with 0.01 *N* NaOH (CO_2 free). The change in pH as a function of the titrant delivered was monitored by a pH meter[7] equipped with a glass electrode.

The pKa values were estimated to be 3.2 (amine group) and 7.1 (hydrazine group).

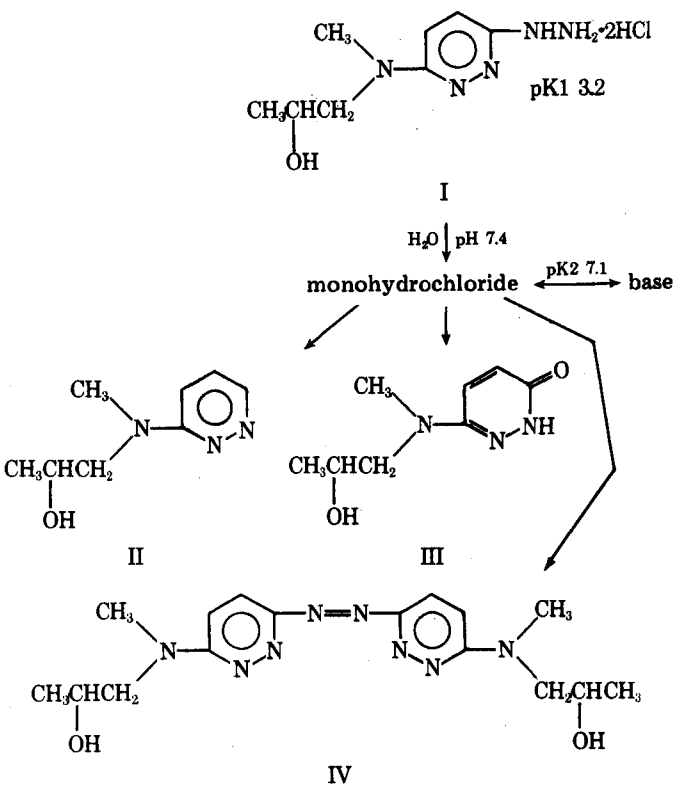
Hydrolytic Degradation—A solution of 2 g (7.4 mmoles) of I in 2 liters of pH 7.4 phosphate buffer was incubated in daylight at 26°. The degradation of I was monitored by UV detection at 244 nm, and the incubation was stopped after 14 days when the maximum absorbance was obtained. The aqueous solution was evaporated *in vacuo*, and the residue was dried over phosphorus pentoxide, triturated with methanol, and filtered. The solvent was removed, and the resulting syrupy liquid was passed through a silica gel column using methanol–chloroform (2:98) as the eluent. The collected fractions were examined by TLC on silica gel plates developed with chloroform–methanol (80:20).

Preparation of Standards—*3-[(2-Hydroxypropyl)methylamino]-pyridazine (II)*—To a solution of 2 g of 3-chloro-6-[(2-hydroxypropyl)methylamino]pyridazine (1) in 40 ml of methanol was added 200 mg of 5% palladium-on-charcoal. The reduction was performed at room temperature under hydrogen (1.3 atm). The catalyst was filtered, the solvent was evaporated, and the residue was treated with alkali and extracted with chloroform.

The extract was dried and the solvent was evaporated to give an oil. This oil was distilled at 150°/0.8 mm Hg to yield 0.84 g (51%) of a pale-yellow oil; IR (liquid film): 3350 (OH) and 1595, 1500, and 805 (pyridazine moiety) cm^{-1}; UV (methanol): maxima at 251 and 332 nm; NMR ($CDCl_3$): δ 8.43 (dd, J = 1.5 and 4.5 Hz), 7.2 (dd, J = 4.5 and 9.5 Hz), 6.93 (dd, J = 1.5 and 9.5 Hz, aromatic H), 4.7 (broad OH), 4.19 (m, CH–OH), 3.60 (d, J = 5.3 Hz, N–CH_2), 3.14 (s, NCH_3), and 1.22 (d, J = 6 Hz, $CHCH_3$) ppm; mass spectra (70 ev, 1.5 mamp): *m/z* 167 (23%, M^+), 122 (100, $[M - CH_3CHOH]^+$), 109 (37), and 94 (26).

Anal.—Calc. for $C_8H_{13}N_3O$: C, 57.46; H, 7.83; N, 25.12. Found: C, 57.59; H, 7.84; N, 25.34.

3-[(2-Hydroxypropyl)methylamino]pyridazine-6-one (III)—A solution of 2 g (10 mmoles) of 3-chloro-6-[(2-hydroxypropyl)methylamino]pyridazine (1) and dry potassium acetate (1.18 g, 0.12 mmole) in 20 ml of acetic acid was refluxed for 4 hr. The solvent was removed *in vacuo*, and the residue was hydrolyzed in boiling 10% HCl for 1 hr. The solution was made alkaline with ammonium hydroxide and evaporated *in vacuo*. The resulting oil was passed through a silica gel column and



Scheme I—Proposed degradation pattern of propildazine in aqueous solution.

[1] Buffers Normex, Carlo Erba, Milan, Italy.
[2] Perkin-Elmer model 177 spectrophotometer.
[3] Beckman DB-GT spectrophotometer.
[4] Perkin-Elmer model R 12 B spectrometer (60 MHz).
[5] Varian MAT model 112 spectrometer.
[6] Silica gel 60-F_{254}, Merck, Darmstadt, West Germany.
[7] Corning model 7.

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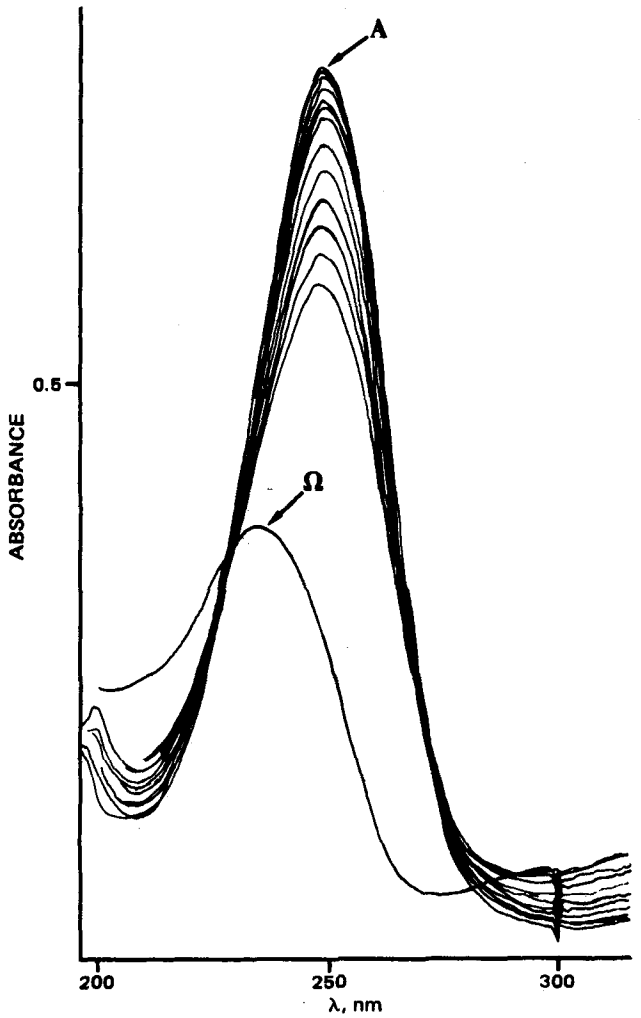

Figure 1—*Representative UV absorption spectra of propildazine (pH 7.4, 3.7 × 10⁻⁵ M, 26°) at different times. Key: A, t = 0; and Ω, 10 times the half-life.*

eluted with chloroform–methanol (95:5) to give 0.46 g (25%) of III as a white powder, mp 152–154°, from ether–methanol (90:10); IR (mineral oil): 3260 (OH), 1685 (C=O), and 1600, 1550, and 843 (pyridazine moiety) cm^{-1}; UV (water): maxima at 245 and 352 nm; NMR (dimethyl sulfoxide-d_6): δ 12.00 (NH), 7.47 and 6.75 (*AB*q, J_{AB} = 10.2 Hz, aromatic H), 4.70 (d, J = 4.7 Hz, CH-*OH*), 3.87 (m, *CH*OH), 3.22 (d, J = 6 Hz, N-CH_2), 2.93 (s, NCH_3), and 1.05 (d, J = 6 Hz, $CHCH_3$) ppm; mass spectra (70 ev, 1.5 mamp): *m/z* 183 (17%, M^+), 138 (100, [M − $CH_3CHOH]^+$), 125 (5), and 110 (22).

Anal.—Calc. for $C_8H_{13}N_3O_2$: C, 52.44; H, 7.15; N, 22.93. Found: C, 52.45; H, 7.17; N, 22.89.

3 - [(2 - Hydroxypropyl)methylamino]-6-isopropylidenehydrazinopyridazine Hydrochloride (V)—A suspension of I (2.7 g, 10 mmoles) in 50 ml of ethanol was stirred with sodium bicarbonate powder (1.68 g, 20 mmoles) under nitrogen at room temperature for 2 hr. Salts were removed, and the solvent was evaporated *in vacuo*. The resulting syrupy liquid was treated with acetone (15 ml) and cooled to yield 1.85 g (68%) of V as a yellow powder, mp 115–120° dec.; IR (mineral oil): 3420 (OH), 3350 (NH), 1645, 1570, 1520, and 830 (pyridazine moiety) cm^{-1}; UV (methanol): maxima at 368 and 280 nm; NMR (dimethyl sulfoxide-d_6): δ 8.00 (s, aromatic H), 3.95 (m, CH), 3.43 (d, J = 5.3 Hz, N-CH_2), 3.08 (s, N-CH_3), 2.10 and 2.07 [s, $C(CH_3)_2$], and 1.09 (d, J = 6.0, CH-CH_3) ppm; mass spectrum (70 ev, 1.5 mamp): *m/z* 237 (16%, M^+), 222 (100, [M − $CH_3]^+$), and 192 (28, [M − $CH_3CHOH]^+$).

Anal.—Calc. for $C_{11}H_{19}N_5O{\cdot}HCl$: C, 48.26; H, 7.36; Cl, 12.95; N, 25.58. Found: C, 48.31; H, 7.37; Cl, 12.92; N, 25.54.

Degradations—The aqueous solutions of I (3.7 × 10^{-5} *M*, 3.7 × 10^{-4} *M*, and 3.7 × 10^{-3} *M*) were prepared in phosphate (pH 6.0 and 7.4) and borate (pH 9.0) buffers. The ionic strength was adjusted to 0.13 with potassium chloride. The reaction flasks were kept in a constant-temperature water bath in the dark at 37 or 26°. Aliquots of the solution were withdrawn periodically, and the UV spectra were scanned from 350 to 200 nm. The methanolic solutions of I (1.85 × 10^{-5} *M*, 3.7 × 10^{-5} *M*, and 3.7 × 10^{-4} *M*) were prepared in 1 *N* methanolic HCl, in methanol, and in 0.9 *M* methanolic trimethylamine and maintained in the dark at room temperature. The degradative behavior of these solutions was verified by following the change of their UV spectra as a function of time.

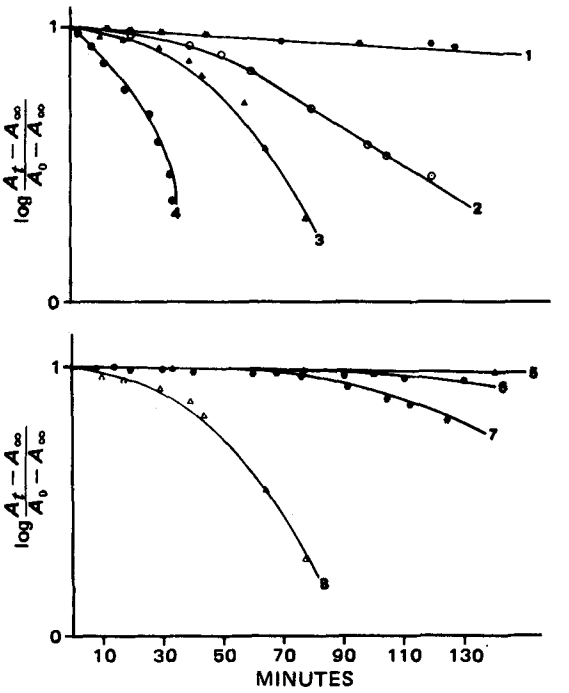

Figure 2—*Profiles of logarithmic normalized absorption (254 nm) versus time. Top: Degradation at different oxidative and pH conditions (c 3.7 × 10^{-5} M, 37°). Key: 1, anaerobic conditions, pH 7.4; 2, aerobic conditions, pH 6.0; 3, aerobic conditions, pH 7.4; and 4, aerobic conditions, pH 9. Bottom: Degradation at different concentrations and temperatures (aerobic conditions, pH 7.4). Key: 5, c 3.7 × 10^{-3} M, 37°; 6, c 3.7 × 10^{-4} M, 37°; 7, c 3.7 × 10^{-5} M, 26°; and 8, c 3.7 × 10^{-5} M, 37°.*

RESULTS AND DISCUSSION

Three main degradation products, A–C, were isolated on column chromatography (Scheme I).

Compound A—The R_f value (0.51) of this oily compound and the IR, UV, NMR, and mass spectra were identical with those of the synthetic standard II.

Compound B—The isolated product had an R_f 0.22, and the melting point was undepressed (150–153°) in admixture with the synthetic sample III. The spectral data were consistent with the assigned structure.

Compound C—After the separation of B, the eluate was concentrated, and the oily residue was crystallized from chloroform–methanol to afford a bright-yellow product, mp 219–223°, R_f 0.24.

Anal.—Calc. for $C_{16}H_{24}N_8O_2$: C, 53.32; H, 6.71; N, 31.08. Found: C, 53.38; H, 6.72; N, 31.02.

The spectra in the UV and visible high region showed an absorption maximum at 420 nm, which shifted to 365 nm after catalytic reduction with palladium-on-charcoal at room temperature and normal pressure. The IR spectra exhibited bands at 3380 cm^{-1} (OH stretching) and at 1590, 1490, 845, and 837 cm^{-1}, characteristic of a substituted pyridazine ring. Moreover, the proton resonances in dimethyl sulfoxide solution confirmed the presence of the same side chain already observed in I and II, while the *AB* quartet at 7.84 and 7.33 (J = 10 Hz) was consistent with a 3,6-disubstituted pyridazine structure.

The mass spectrum indicated the presence of a molecular ion at *m/z* 362, corresponding to the reduced hydrazo derivative of IV. This result may be due to reduction of IV by moisture in the mass spectrometer source (13). When deuterium oxide was introduced, peaks at *m/z* 363 and 364 were observed contemporaneously; the fragmentation pattern with peaks at *m/z* 332 and 278 ruled out isotopic substitution on the side chain. All of these data and the elemental analysis were in agreement with the unusual proposed structure bis-3-[6-(2-hydroxypropyl)methylaminopyridazine]diazene (IV).

Propildazine degradation in water was monitored by following the change in absorbance (A) at 254 nm as a function of time. Two isosbestic points at 233 and 279 nm were observed (Fig. 1). The logarithms of the normalized difference in the absorbance ($A_t - A_\infty / A_0 - A_\infty$, A_∞ being calculated after about 10 half-lives) were plotted against time (Fig. 2). The resulting profiles are clearly concentration (curves 5, 6, and 8), pH (curves 2–4), and temperature (curves 7 and 8) dependent. Moreover, the behavior at pH 7.4, using water bubbled with nitrogen and maintained under nitrogen, showed that oxygen also plays a significant role in the hydrolytic degradation of I (curves 1 and 3).

Propildazine is stable in acidic methanolic solution (0.1 N methanolic HCl, λ_{max} 254 nm unvaried after 2 days), while it is rapidly degraded in methanolic trimethylamine. Moreover, in methanol concentrations of 10^{-5} M, I showed a UV spectrum that rapidly changed its maximum from 259 to 280 nm. This variation was due to trace amounts of acetone present in the spectroscopic grade methanol which reacted with I. In fact, I is a very strong carbonyl group scavenger, giving rise to the corresponding hydrazone 3-isopropylidenehydrazine-6-[(2-hydroxypropyl)methylamino]pyridazine (V) (see *Experimental*).

It is worthwhile to remember the importance of *in vivo* hydrazone formation (14, 15) to explain the prolonged antihypertensive effect of hydralazine-like compounds.

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High-Pressure Liquid Chromatography of Nadolol and Other β-Adrenergic Blocking Drugs

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Abstract □ A high-pressure liquid chromatographic assay was developed for the analysis of the β-adrenergic blocking agent nadolol as a bulk material or formulated in a tablet. Other β-adrenergic blocking drugs such as acebutolol, alprenolol, atenolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, sotalol, and timolol can be chromatographed in this system. An ethylsilane column and a mobile phase consisting of 35% methanol–65% aqueous 0.0005 M hydrochloric acid–0.05 M sodium chloride are used. Detection is either at 254 nm with a fixed UV wavelength detector or at the peak maximum of 220 nm with a variable-wavelength detector. As exemplified by nadolol, the drug content can be quantitated with or without atenolol as an internal standard.

Keyphrases □ Nadolol—high-pressure liquid chromatographic analysis □ β-Adrenergic blockers—high-pressure liquid chromatographic analysis □ High-pressure liquid chromatography—analysis, nadolol and 10 other β-adrenergic agents

β-Adrenergic blocking drugs are presently being used for the treatment of diseases characterized by excess sympathetic nervous activity (1). These illnesses range from cardiac arrhythmia, sinus tachycardia, angina pectoris, hypertension, and migraine to anxiety, essential tremor, alcoholism, and drug dependence.

A high-pressure liquid chromatographic (HPLC) assay was developed for nadolol, *cis*-5-[3-((1,1-dimethylethyl)amino)-2-hydroxypropoxyl] - 1,2,3,4 - tetrahydro-2,3-naphthalenediol, a potent β-adrenergic blocking drug, both as bulk material and in tablets. The chromatographic system also elutes other β-adrenergic blocking agents such as acebutolol, alprenolol, atenolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, sotalol, and timolol at convenient retention times. An analytical profile on nadolol was published previously (2). Nadolol was determined in tablets using colorimetry (3) and in serum using GLC–mass spectrometry (4) or fluorometry (5).

The reported chromatographic procedures are mainly for β-adrenergic blocking agents in body fluids: propranolol in plasma (6–11), atenolol in plasma (12, 13), alprenolol in serum (14), oxprenolol in blood or plasma (15, 16) and in liver tissue (17), acebutolol in serum (18), and practolol in plasma and urine (19). Presumably, these methods could be modified for bulk and formulated material.

EXPERIMENTAL

Materials—Acebutolol[1], alprenolol[2], atenolol[3], metoprolol[4], nadolol[5], oxprenolol[4], pindolol[6], practolol[7], propranolol[5], sotalol[8], and timolol[9] were

[1] May and Baker.
[2] Astra Pharmaceuticals.
[3] Stuart Pharmaceuticals.
[4] Ciba-Geigy.
[5] E. R. Squibb and Sons.
[6] Sandoz Pharmaceuticals.
[7] Ayerst Laboratories.
[8] Mead Johnson.
[9] Merck & Co.

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