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Decomposition of Amitriptyline Hydrochloride in Aqueous Solution: Identification of Decomposition Products

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Abstract □ The decomposition of amitriptyline hydrochloride upon autoclaving in a buffered solution (pH 6.8) was investigated. Three major decomposition products [3-(propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene, dibenzosuberone, and 3-(2-oxoethylidene)-1,2:4,5-dibenzocyclohepta-1,4-diene] were detected and identified by chromatographic and spectroscopic techniques. Evidence is presented that the latter two compounds are formed by further oxidation of 3-(propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene, and a possible decomposition pathway is outlined.

Keyphrases □ Amitriptyline hydrochloride—identification of decomposition products, aqueous solution, mechanisms □ Decomposition—amitriptyline hydrochloride in aqueous solution, identification of products, mechanisms □ Antidepressants—decomposition of amitriptyline hydrochloride in aqueous solution, identification of products, mechanisms

The antidepressant drug amitriptyline hydrochloride may be formulated as a parenteral solution and sterilized by filtration according to BP 1974. Although Henwood (1) showed that amitriptyline base breaks down into dibenzosuberone and Bouche (2) indicated that anthraquinone was formed when the base was boiled in alkaline potassium permanganate solution, there are little published data concerning the stability of the drug.

A project was initiated to assess the drug's instability in aqueous solution and to identify the decomposition products produced during autoclaving and long-term storage. Preliminary experiments showed that decomposition occurred when solutions of amitriptyline hydrochloride in water or phosphate buffers were autoclaved for 30 min at 115–116° in the presence of excess oxygen. The purpose of this paper is to describe the isolation of the decomposition products by TLC and GLC and their identification using mass spectrometry, NMR, and other spectroscopic techniques.

EXPERIMENTAL

Materials—The following chemicals were used: amitriptyline hydrochloride[1], dibenzosuberone[2], ethyl iodide[3], potassium chloride[4], potassium dihydrogen phosphate[4], ether[4], *n*-propanol[4], carbon tetrachloride[4], ethylene dichloride[4], benzene[4], hexane[4], chloroform[4], ethanol[4], methanol[4], ethyl acetate[4], and acetone[4].

Degradation of Amitriptyline Hydrochloride Solution—A 0.5% (w/v) amitriptyline hydrochloride solution was prepared in 0.2 *M* phosphate buffer solution (pH 6.8); 2-ml quantities were distributed in 10-ml ampuls to ensure excess oxygen and sealed. The ampuls were autoclaved at 115–116° for up to 6 hr to produce sufficiently large quantities of the degradation products for subsequent identification by various physicochemical techniques.

TLC—The plates (20 × 20 cm) were coated with silica gel G[5], 250 μm thick, and activated by heating at 110° for 2 hr. They were stored in a desiccator prior to use.

The contents of a heated ampul were extracted with 200 μl of ether, and 50 μl was spotted on a plate in 10-μl portions. Ascending development was carried out over 15 cm with benzene–carbon tetrachloride (7:3 v/v). The spots were detected visually in transmitted light and also by spraying with 4% (v/v) formaldehyde solution BP in concentrated sulfuric acid and viewing under UV light. Dragendorff reagent and 0.4% (w/v) 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid were also used as spray reagents to detect nitrogen- and carbonyl-containing compounds, respectively. The R_f values were determined for the various degradation products (Table I).

[1] Merck Sharp and Dohme.
[2] Authentic specimen, British Pharmacopoeia Commission.
[3] Koch Light Ltd.
[4] Analar grade reagent, B.D.H. Ltd.
[5] Type 60, E. Merck, Darmstadt, West Germany.

Table I—TLC Data for Amitriptyline Hydrochloride Degradation Products and Reference Compounds

Compound	R_f Value	Color Reaction: 4% Formaldehyde Solution BP in Concentrated Sulfuric Acid, Daylight	Color Reaction: 4% Formaldehyde Solution BP in Concentrated Sulfuric Acid, UV Light	Color Reaction: 2,4-Dinitrophenylhydrazine Reagent, Daylight
A	0.70	Brown	—	—
B	0.33	Yellow[a]	Yellow fluorescence	Light orange
C	0.17	Brown	—	Orange
Synthesized I	0.70	Brown	—	—
Dibenzosuberone	0.33	Yellow[a]	Yellow fluorescence	Light orange

[a]The yellow color was not evident at low concentrations, although even low concentrations fluoresce under UV light.

TLC was also carried out in a similar manner using the solvent systems listed in Table II.

To obtain sufficient quantities of the degradation products, 20 ml of autoclaved solution was extracted with two 5-ml quantities of ether. The volume of ether was reduced by evaporation under nitrogen to 2 ml, and this sample was streaked on five plates and developed with benzene–carbon tetrachloride (7:3 v/v). Subsequently, each plate was partially covered by a screen so that only approximately 1 cm was exposed. This area was sprayed to reveal the position of the degradation products, and the nonsprayed areas were removed and eluted separately with methanol. These solutions were evaporated under nitrogen to obtain the degradation products.

GLC—The contents of a heated ampul were extracted with 1 ml of ether, and 5 μl was analyzed using a chromatograph equipped with a flame-ionization detector[6]. A 1.5-m (5-ft) × 0.6-cm (0.25-in.) i.d. glass column, packed with 3% (w/w) OV-25 on 80–100-mesh Chromosorb W AW/DCMS, was used at 220°. The flow rates of hydrogen, air, and nitrogen were 83, 450, and 28.5 ml/min, respectively. Peak assignment was achieved by injecting ethereal solutions of each degradation product separated by TLC.

Spectroscopic Analysis—The UV spectra[7] of solutions of the isolated degradation products in methanol were obtained using 1-cm path length silica cells.

To obtain IR spectra[8], samples were presented in the form of liquid films or dilute solutions in carbon tetrachloride.

NMR spectra[9] were obtained by preparing solutions of the products in deuterated chloroform containing tetramethylsilane as the internal marker.

Mass spectra[10] were obtained using a beam energy of 70 ev with the sample probe maintained at 210°.

Synthesis of 3-(Propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene (I)—Amitriptyline hydrochloride (500 mg) was made alkaline with 1 *N* sodium hydroxide and extracted with ether. The extract was dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated to dryness *in vacuo*, and 10-ml volumes of ethyl iodide and ether were added. The mixture was refluxed on a steam bath for 20 min, and the unreacted reagents were removed by distillation. Then the residue was dried in a stream of dry nitrogen and dissolved in a minimum of methanol, and excess silver oxide was added.

The mixture was shaken and filtered, and the filtrate was sealed in ampuls under nitrogen and autoclaved for 5 hr. The solvent was removed, and the residue was dissolved in 10 ml of ether and extracted with 5 ml of 5 *N* hydrochloric acid. The ethereal layer was washed with a further two 5-ml volumes of acid, dried over anhydrous sodium sulfate, and filtered, and the product was isolated by vacuum evaporation. The purity of the compound was checked by TLC.

The physicochemical data on the compound were as follows: UV (methanol): λ_{max} 268 nm; IR (liquid): 3060 (aromatic CH stretch), 2920 (CH_2 stretch), 1650 (diene), 910 and 990 (—CH=CH_2), and 755 and 765 (aromatic hydrogen) cm^{-1}; NMR: δ 3.2 (unresolved multiplet, protons on seven-membered ring), 5–5.5 (multiplet, methylene protons), 6.2–6.7 (multiplet, methine protons), and 7.2 (multiplet, aromatic protons) ppm.

The mass spectrum showed a molecular ion at *m/e* 232 (100%) corresponding to $C_{18}H_{16}$ and significant peaks at 231 (34), 229 (8), 218 (11), 217 (65), 216 (26), 215 (52), 204 (18), 203 (23), 202 (36), 191 (10), 189 (16), 151 (8), 142 (17), 116 (24), 113 (13), 106 (17), 105 (13), 104 (8), 92 (8), 88 (7), and 49 (7).

Anal.—Calc. for $C_{18}H_{16}$: C, 93.11; H, 6.89. Found: C, 92.85; H, 7.14.

RESULTS AND DISCUSSION

TLC of degraded amitriptyline hydrochloride, using benzene–carbon tetrachloride (7:3 v/v), indicated the presence of three major decomposition products (A, B, and C). Table I gives their R_f values and color reactions. Also included in Table I are the data for the synthesized I and dibenzosuberone (II). The chromatographic behavior of A and B and the two reference compounds in a range of developing solvent systems is shown in Table II.

GLC produced peaks that correspond to amitriptyline, A, and B. Product C could not be detected in the extract, but a peak was obtained when injecting the eluate from thin-layer plates. Table III shows the retention times of these products together with those of the reference compounds.

From the chromatographic results in Tables I–III, it is apparent that Product A was I. This finding was confirmed by the similarity of their UV, IR, NMR, and mass spectra.

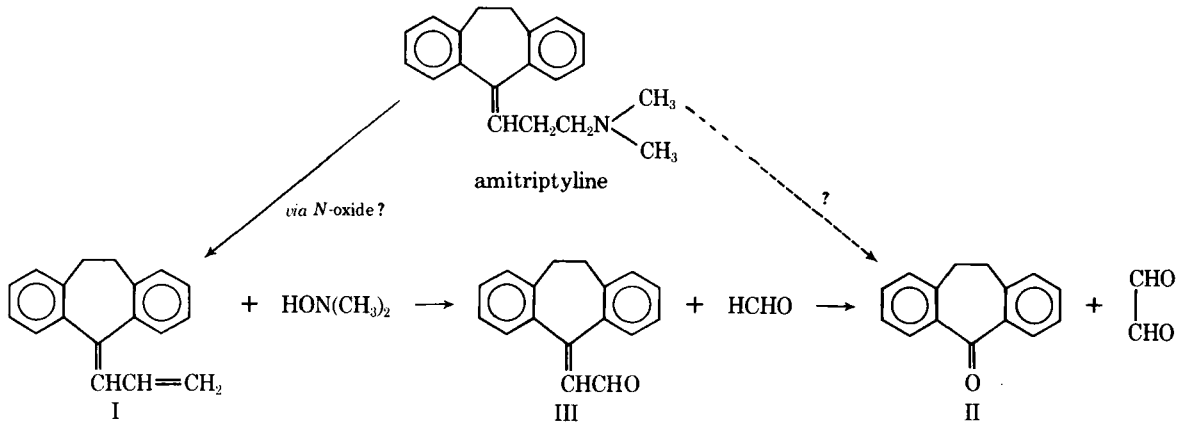
When chromatograms of A, produced by development with benzene–carbon tetrachloride (7:3 v/v), were exposed to air for 12 hr and then developed at right angles to the direction of initial development with chloroform–benzene–diethylamine (5:4:1 v/v), the decomposition product was broken down into B and C.

The identity of B was established by comparing its physicochemical data with those of dibenzosuberone. As shown in Tables I–III, their chromatographic data were identical, as were their UV spectra (λ_{max} 270 nm). Final confirmation that B was dibenzosuberone was obtained by comparison of their mass spectra. These spectra were identical, with a molecular ion at *m/e* 208 (100%) corresponding to $C_{15}H_{12}O$ and significant peaks at 207 (43), 192 (7), 181 (7), 180 (45), 179 (48), 178 (36), 177 (5), 166 (7), 165 (24), 151 (7), 150 (5), 90 (7), 88 (24), 77 (7), 76 (2), 75 (17), 62 (10), 50 (7), 49 (5), and 39 (7).

Table II—Chromatographic Behavior of A, B, Dibenzosuberone, and Synthesized I

Solvent System	R_f Value: Product A	Synthesized I	Product B	Dibenzosuberone
Benzene–hexane (3:2 v/v)	0.77	0.77	0.27	0.27
Ethylene dichloride–hexane (1:1 v/v)	0.83	0.83	0.37	0.37
Chloroform–benzene (1:4 v/v)	0.77	0.77	0.48	0.48
Ethyl acetate–carbon tetrachloride (3:2 v/v)	0.73	0.73	0.69	0.69
n-Propyl alcohol–benzene–carbon tetrachloride (3:3:4 v/v)	0.93	0.93	0.91	0.91
Ethanol–water (3:1 v/v)	0.75	0.75	0.75	0.75

[6] Pye 105 gas chromatograph.
[7] Pye-Unicam SP1800 spectrophotometer.
[8] Perkin-Elmer 157G spectrophotometer.
[9] Varian A60 spectrometer.
[10] A.E.I. MS 902 mass spectrometer.



Scheme I—Proposed decomposition pathway for amitriptyline in aqueous solution

Table I shows that C reacted with 2,4-dinitrophenylhydrazine, and this finding indicated the presence of a carbonyl function. The physicochemical data for the compound were as follows: UV (methanol): λ_{max} 280 nm; IR (liquid): 3020 (aromatic CH stretch), 2920 and 2860 (CH_2 stretch), 1675 (—C=O in conjugated system =CH—CHO), and 767 and 757 (aromatic hydrogen) cm^{-1}; NMR: δ 3.3 (unresolved multiplet, protons on seven-membered ring), 4.4–4.6 (triplet, methine protons), 7.3 (multiplet, aromatic protons), and 9.6–9.7 (aldehydic proton) ppm.

The mass spectrum showed a molecular ion at *m/e* 234 (100%) corresponding to $C_{17}H_{14}O$ and significant peaks at 233 (69), 232 (7), 219 (17), 206 (20), 205 (23), 204 (10), 203 (20), 202 (23), 193 (7), 192 (7), 191 (20), 190 (7), 189 (13), 178 (13), 177 (7), 164 (10), 151 (10), 128 (10), 115 (10), 109 (10), 91 (7), 69 (17), 57 (7), 55 (7), 43 (7), and 41 (7).

From these data, it was concluded that C was 3-(2-oxoethylidene)-1,2:4,5-dibenzocyclohepta-1,4-diene (III).

The formation of dibenzosuberone upon autoclaving amitriptyline hydrochloride could be explained by direct oxidation of the olefinic double bond of the drug. However, such a mechanism does not account for the production of I and III, since only portions of the side chain have been lost. Cope *et al.* (3) showed that loss of dialkyl amino function can proceed *via* formation of *N*-oxide. Although it has not yet been possible to isolate an *N*-oxide in the degraded sample, it seems that *N*-oxidation, followed by loss of dimethylhydroxylamine, is the most probable route of decomposition.

Table III—GLC Behavior of Degradation Products of Amitriptyline Hydrochloride and Reference Compounds

Compound	Retention Time, min
A	3.5
B	4.5
C	8.0
Dibenzosuberone	4.5
Synthesized I	3.5
Amitriptyline	7.0

The aldehyde (III) has been shown to result from the further oxidation of I. The formation of formaldehyde was also detected in this step. A quantity of the isolated allylic compound (I) was placed in a conical flask having a sealed vapor trap (containing chromotropic acid in sulfuric acid) connected to the neck. Upon storage, the chromotropic acid solution developed a violet color, indicating the presence of formaldehyde.

During the subsequent oxidation of III to dibenzosuberone, glyoxal was formed. It was detected by adding excess *o*-aminophenol and calcium oxide to an ethanolic solution of aldehyde, which had been allowed to stand overnight. A positive brick-red color, indicative of glyoxal, was produced.

It is evident that oxidation of the olefinic double bond of amitriptyline hydrochloride is not necessarily the first step in the degradation of the drug; Scheme I shows the proposed decomposition pathway.

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