xanthones, those containing hydroxyl groups at 1,3- and 6- or 8-positions were more active than those containing other substituents (methoxy or glucosyloxy) at these positions.

Norswertianolin (1-glucosyloxy-3,5,8-trihydroxyxanthone) (XXIII) and swertianolin (1-glucosyloxy-3-methoxy-5,8-dihydroxyxanthone) (XXIV), which occur in a number of *Swertia* species (9–11), were previously reported to produce antitubercular activity, although their exact potency was not reported (11). In the present investigation, XXIII and XXIV exhibited only a weak anti-*M. tuberculosis* activity. The aglucone (XX) of XXIII appeared to be more active than the corresponding 1-*O*-glucosyl derivative, suggesting the importance of the free hydroxyl group at the 1-position for activity.

Hansch analysis (12) with the partition coefficients (π) and MIC values of I–XXIV was attempted but did not provide a good correlation. Electronic and steric factors also seemed to be responsible for the observed biological activity.

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Characterization of Products Derived from Aprindine Hydrochloride Photolysis

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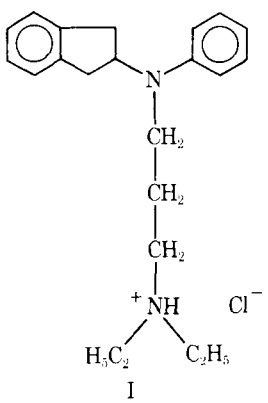
Received March 7, 1977, from the *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206.* Accepted for publication August 29, 1977.

Abstract □ An investigation of the products resulting from the photolysis of aprindine hydrochloride is described. The compounds were characterized by GLC spiking experiments and combined GLC–mass spectrometry. In some cases, R_f values derived from TLC and/or preparative TLC and subsequent high-resolution mass spectral measurements also were employed for identification.

Keyphrases □ Aprindine hydrochloride—photolysis products identified by GLC and combined GLC–mass spectrometry □ GLC and combined GLC–mass spectrometry—identification of photolysis products of aprindine hydrochloride □ Photolysis—aprindine hydrochloride, products identified by GLC and GLC–mass spectrometry □ Cardiac depressants—aprindine hydrochloride, photolysis products identified by GLC and GLC–mass spectrometry

In a previous report (1), a GLC method was presented which effectively monitors the stability of the antiarrhythmic agent aprindine hydrochloride (2) [*N,N*-diethyl-*N'*-(2-indanyl)-*N'*-phenyl-1,3-propanediamine hydrochloride] (I). During this work, it was discovered that I is not stable to UV irradiation, as evidenced by extra peaks in the gas chromatograms obtained from GLC analysis of the photolysis solutions. Tentative identifications were suggested on the basis of GLC retention times, but detailed characterizations were not reported.

This report describes the characterization of the aprindine photoproducts *via* TLC, GLC, and combined GLC–mass spectrometry.



I

EXPERIMENTAL

GLC and combined GLC–mass spectrometric measurements were obtained using the instruments previously described (1). For this work, however, the combined gas chromatograph–mass spectrometer was interfaced to a data system[1] that provided a background subtraction routine as an option. Additionally, nonlinear temperature programming provided optimal resolution.

A typical run was carried out as follows: the oven was equilibrated at 120°, the sample was injected, and the chromatogram was recorded isothermally for 3.0 min. The oven temperature was then raised to 190°.

[1] The 150 data system, Systems Industries, Sunnyvale, Calif.

Table I—Chromatographic and Mass Spectral Data

GLC Component	Retention Time, min	Percent[a]	R_f[b]	Principal Ions (*m/e*)
1	0.3	0.5	—	—[c]
2	0.5	2.2	—	—[c]
3	1.3	3.7	—	91 ($C_7H_7^+$), 106 (M − HCN), 116 (M − NH_3), 133 (M^+)
4	2.2	—[d]	—	91 ($C_7H_7^+$), 105 (M − CH_2=NCH_3), 117 (M − CH_3NH·), 118 (M − CH_2=NH), 147 (M^+)
5	5.2	10.7	0.43	Series 1, 72, 86, 113; Series 2, 93, 106, 119, 120, 133, 134; 206 (M^+)
6	7.0	4.4	—	—[c]
7	7.5	5.8	0.12	Series 1, 73, 86, 100, 113; Series 2, 130, 158, 173; 246 (M^+)
8	7.9	6.0	0.84	77 ($C_6H_5^+$), 93 ($C_6H_5NH_2^{7+}$), 104 (133 − HCN), 106 (133 − CH_2=NH), 133 (M − C_6H_5·), 209 (M^+)
9	8.2	3.8	—	—[c]
10	14.6	9.6	0.67	Series 1, 58, 72, 86; Series 2, 208, 222, 236, 249; 116 ($C_9H_8^{7+}$), 178 (M − indan), 192 (M − C_8H_6), 294 (M^+)
11	17.5	53.3	0.51	—[c]
12	18.6	—[d]	—	—[c]

[a] Calculated from the chromatogram using peak area (height × width at half height). [b] Methanol–acetic acid (8:2) on silica gel. [c] See text. [d] Trace component.

After an additional 7.0 min, the temperature was raised again to 210°. The run was completed after 10 min at the latter setting. A similar procedure but a wider temperature range, 80–240°, provided the best resolution with the combined gas chromatograph–mass spectrometer.

The photolyses were run in methanol solution using a 250-ml photochemical reactor[2] equipped with a medium pressure 200-w mercury vapor immersion lamp[3], a nitrogen inlet, a cooling jacket, and a condenser. A filter sleeve[4] was fitted over the quartz jacket of the immersion unit to exclude all wavelengths below 220 nm. The solutions (typically 2.0 g in 200 ml) were purged with nitrogen for 30 min prior to irradiation and agitated during irradiation by a stream of nitrogen bubbles generated through a circular perforated glass tube placed on the bottom of the solution chamber.

At the conclusion of the photolyses (30.5 hr), the solution was concentrated on a rotary evaporator to one-tenth of its original volume. Aliquots of the concentrate were made alkaline with excess 10% sodium carbonate and extracted into chloroform. The chloroform extracts were employed in all subsequent work.

TLC was employed using 0.25-mm (for the determination of R_f values) and 0.50-mm (for preparative work) thick silica gel plates[5]. The analytical solvent system was methanol–acetic acid (8:2)[6], and the preparative system was ethyl acetate–triethylamine (95:5). The thin-layer plates were visualized by spraying with the Folin–Ciocalteu reagent (3) followed by 20% aqueous sodium carbonate. This procedure yields blue to blue-purple spots on a white background[6].

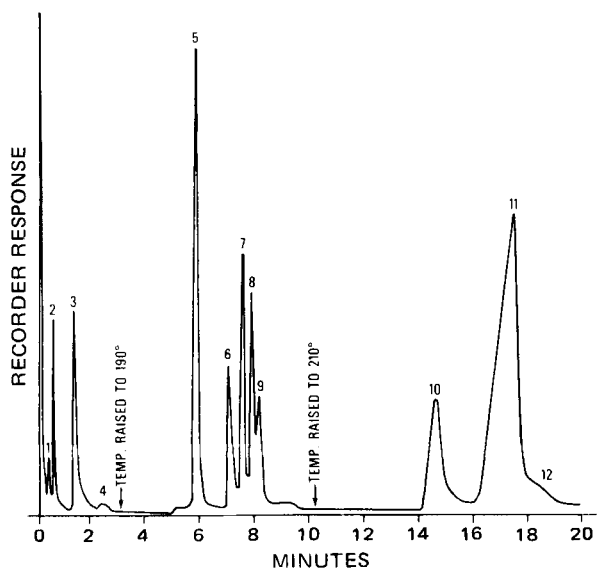


Figure 1—*Gas chromatogram obtained from the photolysis solution.*

[2] Catalog No. 6515-03, Ace Glass Inc., Vineland, N.J.
[3] Catalog No. 6515-32, Ace Glass Inc., Vineland, N.J.
[4] Catalog No. 6515-40, Vycor 7010 (Corning), Ace Glass Inc., Vineland, N.J.
[5] Silica gel 60 F_{254} plates, E. Merck, Darmstadt, West Germany.
[6] B. S. Rutherford and R. H. Bishara, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46206, unpublished work.

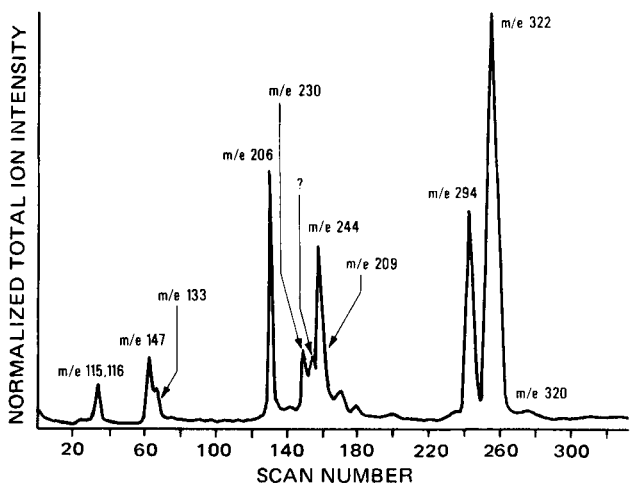


Figure 2—*Total ion intensity plot and molecular ions of the components derived from photolysis.*

Substances were isolated from the thin-layer plates by scraping off the appropriate zones, grinding them to a fine powder in a mortar, and adding the powder to spin thimbles[7] where the material was extracted by centrifugation using acetone–methanol (7:3). Accurate mass measurements[8] were obtained by peak matching at a resolution of 15,000.

All reagents were reagent or analytical grade and were used without further purification. Authentic samples of 2-anilinoindan[9], desethylaprindine[9], *N,N*-diethyl-*N'*-phenyl-1,3-propanediamine[9], and desphenylaprindine[10] were used.

RESULTS AND DISCUSSION

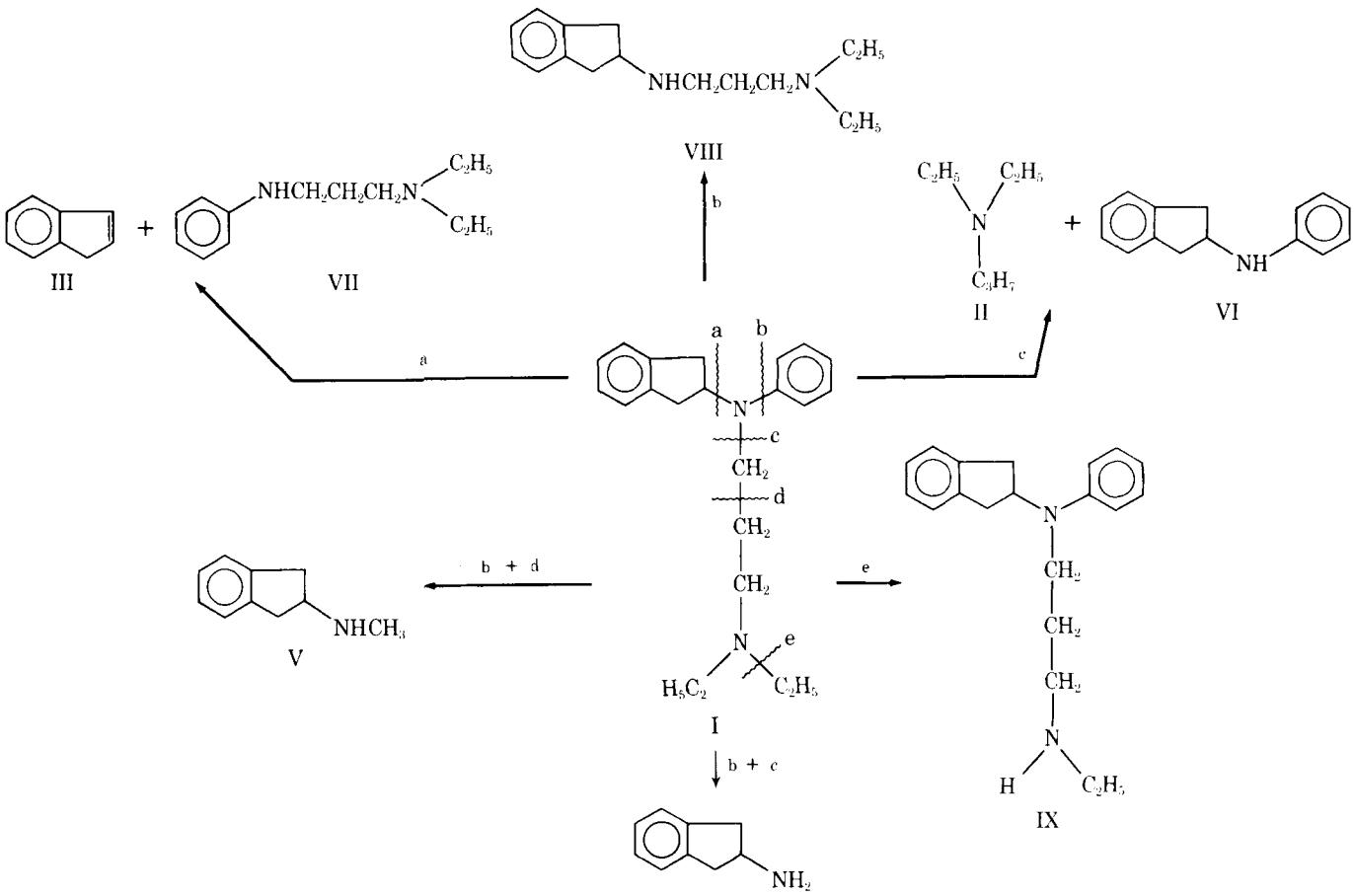
Figures 1 and 2 show the gas chromatogram and total ion plot (TII, normalized total ion intensity, *versus* mass spectral scan number) obtained at the conclusion of a typical photolysis. Some peak distortions occurred in the TII plot relative to the chromatogram. However, concentration–response studies with authentic materials allowed demonstration of the quantitative accuracy of the chromatogram.

The structures of the various components were determined by comparison with mass spectral literature data and with R_f values (TLC), retention times (GLC), and mass spectra of authentic materials.

Components 1 and 2—The mass spectral data for Components 1 and 2 were in excellent agreement with those previously published for *N,N*-diethylpropylamine (II) (4) and indene (III) (5).

Components 3, 4, and 8—The major ions observed in the mass spectra of Components 3 and 4 identified these substances as 2-aminoindan (IV) and 2-methylaminoindan (V), respectively. Mass spectral and chromatographic comparisons with authentic material showed Component 8 to be 2-anilinoindan (VI) (Table I).

[7] Terra-Marine Bioresearch, La Jolla, Calif.
[8] Varian MAT 731 mass spectrometer.
[9] Supplied by Dr. P. J. Murphy, Lilly Research Laboratories.
[10] Supplied by Dr. L. Dodion, A. Christiaens, S.A., Brussels, Belgium.



Scheme I

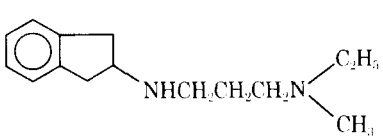
Components 5, 7, 10, and 11—The mass spectra of aprindine hydrochloride and a number of photoproducts showed analogous fragmentation patterns, which served as sensitive structural probes. Typically, Component 11 (aprindine, I) underwent fragmentation at positions α and β to the two nitrogens and the presence of two homologous series of ions (Series 1, *m/e* 72, 86, 100, and 114; and Series 2, *m/e* 222, 236, and 249) indicated these cleavages. In addition, the data suggested that each cleavage occurs in a dual mode such that the positive charge is found on both fragment ions.

Analogous ion series were observed for Components 5, 7, and 10 (Table I) and, together with the chromatographic comparisons with authentic materials and high-resolution mass spectrometry (*vide infra*) on Components 5 and 10, identified these substances as *N,N*-diethyl-*N'*-phenyl-1,3-propanediamine (VII), desphenylaprindine (VIII), and desethylaprindine (IX), respectively.

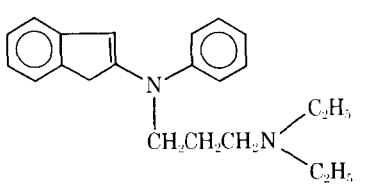
Components 6, 9, and 12—Components 6 and 12 were not positively identified, but the mass spectral data suggested that Component 6 was *N*-methyl-*N*-ethyl-*N'*-indanylpropanediamine (X) and Component 12 was a more highly oxidized analog of I, which is probably best represented by Structure XI.

Component 9 was so poorly resolved in the TII plot that definitive spectra could not be obtained in spite of extensive background subtraction.

Preparative TLC and High-Resolution Mass Spectrometry—Additional characterization data were obtained for Components 5 and 10 by preparative TLC followed by high-resolution mass spectrometry (see *Experimental* section). Measurements on the molecular ion of Component 5 yielded a value of 206.1780 (calc. for $C_{13}H_{22}N_2$: 206.1783). A similar determination for the molecular ion of Component 10 afforded a measured mass of 294.2088 (calc. for $C_{20}H_{26}N_2$: 294.2095).



X



XI

CONCLUSIONS

The photolysis of aprindine resulted almost completely in products reflecting various combinations of C–N bond cleavages (Scheme I). The structural elucidations confirmed the suggestions made previously regarding the identities of two of the photoproducts (1) and showed that the photochemistry observed in this study is analogous to behavior suggested for other tertiary amines (6).

Compounds VI, VIII, and IX also have been shown to be metabolites of aprindine (2, 7, 8).

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Structure–Activity Studies on Mutagenicity of Nitrosamines Using Molecular Connectivity

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Received August 3, 1977, from the **Department of Pharmaceutical Chemistry, Medical College of Virginia, Virginia Commonwealth University and MCV/VCU Cancer Center, Richmond, VA 23298*, and the ‡*Department of Chemistry, Eastern Nazarene College, Quincy, MA 02170.* Accepted for publication September 1, 1977.

Abstract □ The structure–activity relationship of a series of nitrosamines was evaluated for mutagenic potency as measured in the Ames test. The structural description was made using molecular connectivity. A good correlation was found.

Keyphrases □ Nitrosamines, various—mutagenic potency related to molecular connectivity indexes □ Mutagenicity—various nitrosamines, related to molecular connectivity indexes □ Molecular connectivity indexes—various nitrosamines, related to mutagenic potency □ Structure–activity relationships—various nitrosamines, mutagenic potency related to molecular connectivity indexes

The mutagenicity of a molecule is important in assembling a profile of its toxicity and environmental effects. It was stated (1) that there is a correlation between mutagenicity and carcinogenicity, high enough to warrant the routine testing for this property. Such a test is currently used in some laboratories (2).

The compounds are tested on petri plates with mutants of *Salmonella typhimurium*. Homogenates of rat or human liver are added to the plates. The number of revertants per nanomole is determined from dose–response curves and is an indication of the compound's mutagenicity. McCann *et al.* (1), using the Ames test, recently evaluated 300 compounds of various types.

The nitrosamines, known to occur in cigarette smoke, nitrate pickled meat, and smoked fish (3), were studied (1). It was demonstrated (4) with experimental animals that nitrosamines are potent carcinogens.

In this study, previous data (1) on mutagenicity were used as a measure of potency to examine the structural influences on this activity. Fifteen nitrosamines (Table I), tested under identical conditions and having precisely reported data, were considered.

EXPERIMENTAL

To evaluate the structure–activity relationships in this series, a recently developed method, molecular connectivity (5), was used. This method for describing the structure of a molecule has its roots in topology. A series of indexes reflect a weighted count of subgraphs which, from simple calculations, lead to values encoding considerable structural information. The information makes possible the evaluation of structural features influencing physical property values (6) and biological activity (7–9). The use of extended terms (10) and the application of molecular connectivity to heteroatoms (11) make the method ideally suited for a study of the structure–activity relationships of biologically important molecules.

RESULTS

A systematic search of the connectivity indexes for the molecules in Table I revealed two relationships with nearly equal quality. By expressing the activity as the natural log number of revertants per nanomole, the following equations were found:

$$\ln R = 2.398\ (\pm 0.032)\ {}^{0}\chi - 4.095\ (\pm 0.132)\ {}^{1}\chi^{v} - 5.590\ (\pm 1.158) \quad \text{(Eq. 1)}$$

$$r = 0.964 \quad s = 1.09 \quad n = 15 \quad F = 78.8\ (p < 0.02)$$

$$\ln R = 2.946\ (\pm 0.066)\ {}^{2}\chi - 9.090\ (\pm 0.729)\ {}^{4}\chi_{p}{}^{v} - 4.662\ (\pm 1.052) \quad \text{(Eq. 2)}$$

$$r = 0.967 \quad s = 1.05 \quad n = 15 \quad F = 85.2\ (p < 0.02)$$

Both equations predict the ln R to less than 10% of the log range of experimental values in this set. The common structural feature in the set is the nitrosamine group and must be considered to be necessary, but not sufficient, for potency. Subsidiary structural features, quantified by

Table I—Mutagenicity/Carcinogenicity of Nitrosamines

	Compound	ln R[a]	Equation 1, Calc. ln R	Equation 2, Calc. ln R
1	Dipropyl-*N*-nitrosamine	−2.526	−2.573	−2.711
2	Dibutyl-*N*-nitrosamine	−1.897	−3.276	−2.128
3	Dipentyl-*N*-nitrosamine	−2.996	−3.979	−3.747
4	*N*-Nitrosopyrrolidine	−3.912	−3.626	−4.232
5	*N*-Nitrosomorpholine	−2.813	−2.247	−1.857
6	*N*-Nitrosopiperidine	−4.605	−3.977	−4.798
7	*N*-Methyl-*N*-nitroso-*N*′-nitroguanidine	7.226	5.176	5.287
8	*N*-Ethyl-*N*-nitroso-*N*′-nitroguanidine	5.858	4.513	4.020
9	*N*-Propyl-*N*-nitroso-*N*′-nitroguanidine	3.689	4.161	3.791
10	*N*-Butyl-*N*-nitroso-*N*′-nitroguanidine	3.892	3.811	3.959
11	*N*-Isobutyl-*N*-nitroso-*N*′-nitroguanidine	4.344	4.792	5.304
12	*N*-Pentyl-*N*-nitroso-*N*′-nitroguanidine	3.091	3.669	3.264
13	*N*-Hexyl-*N*-nitroso-*N*′-nitroguanidine	1.668	3.317	2.699
14	*N*-Nitrosomethylurea	1.482	1.716	2.676
15	*N*-Nitrosoethylurea	0.095	1.117	1.069

[a] Natural log of revertants per nanomole.