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Thioxanthones: Structural Differences between Lucanthone and Its N-Methyl Derivative

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SUMMARY

Lucanthone (1-diethylaminoethylamino-4-methylthioxanthone) is a bacteriostatic and carcinostatic agent and readily combines with DNA. N^{11}-Methyl substitution results in virtual deletion of these activities. Studies of electronic spectra, vibrational spectra, and ionization constants indicate that N^{11}-methyl substitution precludes coplanarity of the amine side chain and the thioxanthone ring system. The possible relationship of this change in molecular configuration to the strikingly reduced biological and biochemical activities of the N-methyl derivative is discussed.

INTRODUCTION

N^{11}-Methyl substitution of 1-dialkylaminoalkylamino-4-methylthioxanthones (Fig. 1) is generally associated with a marked reduction in bacteriostatic (1) and carcinostatic (2) activity. In addition, the above compositional change is accompanied by a complete loss of the capacities to inhibit RNA polymerase, to stabilize DNA against heat denaturation, and to increase the viscosity of DNA solutions (1, 3). In view of these striking differences in cytotoxic and biochemical action, a detailed comparison of the physicochemical properties of lucanthone (compound I, Fig. 1) and its N^{11}-methyl derivative (compound II, Fig. 1) was undertaken.

Stuart-Briegleb molecular models suggest that the replacement of H by CH_3 on N-11 of lucanthone results in steric interference between the N-methyl group and the oxygen atom on C-9, necessitating a rotation of the amine side chain on the C-1–N-11 bond axis to permit these substituents to attain their approximate van der Waals separation. This rotation implies a change in the orientation of the $2p$ orbital of N-11 relative to those of the ring system, resulting in reduced conjugation across the C-1–N-11 bond and an increased electron density on N-11.

MATERIALS AND METHODS

Materials

The synthesis and properties of lucanthone (Burroughs Wellcome & Company, Inc.) have been described by Mauss (4), and those of the N-methyl derivative, by Elslager, Cavalla, Closson, and Worth (5).

The stable hydrochlorides were recrystallized to constant melting point from appropriate solvents. The melting points obtained agreed closely with the published figures. The free diamines[1] were obtained from alkaline solutions of their hydrochlorides. The hydrochlorides and the corresponding bases were shown to be chro-

[1] The following descriptive terms are used (superscripts refer to circled numbers in Fig. 1): *free diamine*, neither N^{11}- nor N^{14}-protonated; *monohydrochloride*, N^{14}-protonated; *dihydrochloride*, both N^{11}- and N^{14}-protonated.

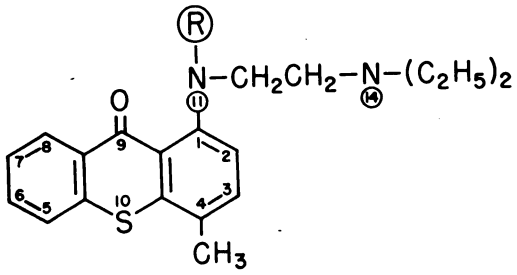

FIG. 1. *Structures of lucanthone (compound I; R = H) and its N^{11}-methyl derivative (compound II; R = CH_3)*

matographically pure on thin layer chromatograms (neutral silica gel with fluorescent indicator) developed in the following solvent systems: methanol; absolute ethanol–*n*-hexane, 1:1; butanol–acetic acid, 9:1; dioxane–acetone–water, 7:7:1; ethanol–ammonia, 9:1–20:1.

Methods

Electronic spectra were determined on a Cary model 15 recording spectrophotometer calibrated for wavelength by the emission spectrum of a mercury arc and for absorbance with standard filters used singly and in combination. Band resolution was confirmed by methods described in "Optimum Spectrophotometer Parameters," Application Report AR 14-2 (Applied Physics Corporation, 1964). Samples were dried at room temperature in a vacuum desiccator over anhydrous calcium chloride and dry paraffin shavings for at least 24 hr prior to spectrophotometry. Spectra were measured in the following solvents: free diamines, in *n*-hexane (spectroscopic quality); monohydrochlorides, in 0.01 M sodium phosphate buffer, pH 6.70; dihydrochlorides, in HCl (H_0 −2.5 and −3.5). In view of the spectral differences described below, the electronic spectrum of the base of N^{11}-methyllucanthone was redetermined following chromatography on silica gel (solvent, ethanol–ammonia, 20:1); the spectral characteristics were unchanged.

Infrared spectra were determined on a Perkin-Elmer model 137 recording spectrophotometer with NaCl optics. Despite the low dispersion of NaCl over the high-frequency portion of the infrared spectrum, satisfactory resolution and reproducibility were attained. Instrument error was held to a minimum by allowing the spectrophotometer to warm up for at least 1 hr prior to each set of determinations (to permit the monochromator to achieve thermal equilibrium), and by calibrating each spectrum with a polystyrene standard.

The ionization constant of lucanthone was determined by a spectrophotometric adaptation of the indicator method of Hammett *et al.* (6–8). Three sets of determinations were made at sample compartment temperatures of 24° ± 1°, 25.5° ± 1.5°, and 27.5° ± 1.5°, using sample concentrations of 1.2–1.5 × 10^{-4} M. The spectra of the monohydrochloride were measured in water and 0.01 M sodium phosphate buffer (pH 6.70), and those of the dihydrochloride, in HCl (H_0 −2.5 to −4.5). Sets of determinations were obtained at fixed wavelengths at the absorption maxima of the mono- and dihydrochlorides over the following acidity function[2] range: H_0 +0.20 to −0.52. Solvents were prepared from standardized HCl solutions. The acidity functions of the dilutions used were confirmed spectrophotometrically, using *o*-nitroaniline as the indicator. The acidity functions thus obtained agreed closely with published figures (7).

The ionization constant of N^{11}-methyllucanthone was measured by standard spectrophotometric methods (10). Determinations were made at sample compartment temperatures of 22° ± 2°, using sample concentrations of approximately 10^{-4} M. The spectra of the monohydrochlorides were obtained in 0.01 M potassium phosphate and potassium borate buffers, pH 5.98–9.13, and those of the dihydrochloride, in 0.04, 0.08, and 0.80 N HCl, pH 1.4, pH 1.1, and H_0 −0.07, respectively. A slight medium effect (see below) was noted at H_0 −0.07. Isosbestic points were found at 374.0 and 425.5 nm. The degree of ionization was determined at fixed wavelength at the absorption maximum of the dihydrochloride in 0.010 M sodium formate buffer over a pH range of 3.11–4.11. pH was measured on a Beckman model G pH meter standardized with Beckman and

[2] The acidity function, H_0, is defined in reference 9; see also references 6 and 7.

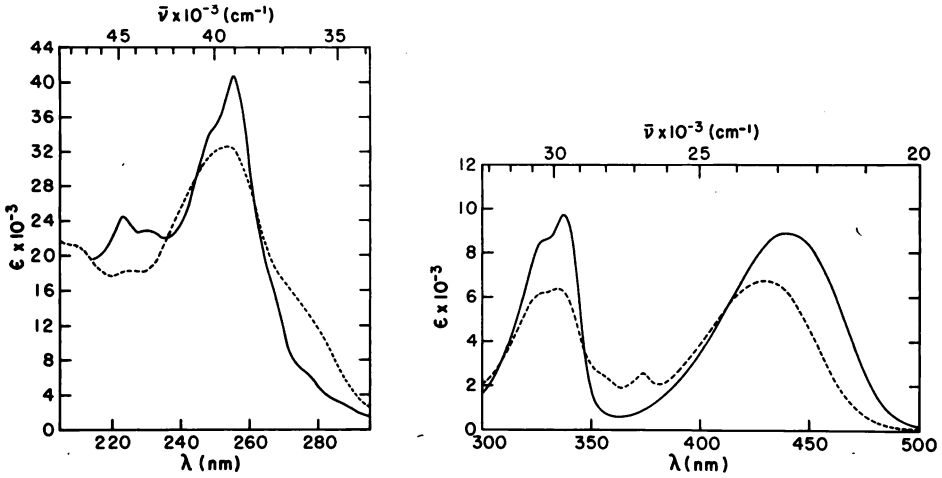

FIG. 2. *Electronic spectra of lucanthone* (——) *and its N^{11}-methyl derivative* (– – –). λ = wavelength; $\bar{\nu}$ = wave number; ϵ = molar absorptivity.

Harleco pH reference buffer solutions: pH (at 25°) 3.00, 3.33, 4.00, 6.86, 7.00, 9.18, and 10.00. Each measurement was immediately preceded by standardization with the appropriate buffer. A minimum of three determinations was made on each test solution.

RESULTS

Electronic Spectra

Electronic spectra of the free diamines of lucanthone and its N^{11}-methyl derivative are depicted in Fig. 2. The introduction of a methyl group on N-11 results in a marked hypochromic effect, hypsochromic spectral shifts, increased bandwidth, and the appearance of a new band at approximately 374 nm. In addition, there is degradation of fine structure in the middle- and high-frequency band complexes. The hypochromic effect and hypsochromic shifts, particularly of the low-frequency band system, are entirely consistent with steric inhibition of resonance (11–19). Broadening of the conjugation bands of sterically hindered compounds has been attributed to a redistribution of the total intensity among various vibrational levels of the electronic excited state (20–22), and to changes in the distribution of molecular conformations in the ground electronic state (20, 21). The above considerations may account for increased bandwidth, small frequency shifts (23), blurring of fine structure (20, 21), and that portion of the reduced absorptivity at the band maxima which is independent of changes in oscillator strength (22).

The electronic spectra of compound II and other N^{11}-methyl-substituted analogues of lucanthone manifest an absorption band at approximately 374 nm (solvent, *n*-hexane) which is not discernible in spectra of compounds in which the base linked to C-1 is a secondary amine. In the spectrum of the monohydrochloride of compound II the 374 nm band merges with the high-wavelength band complex. Spectra of the dihydrochlorides of the two thioxanthones reveal but a single band, of identical intensity and bandwidth, in the visible portion of the spectrum. However, the absorption maximum of N^{11}-methyllucanthone appears at somewhat lower frequency ($\Delta\bar{\nu} \cong 350$ cm^{-1}). The similarity of the spectra of the dihydrochlorides is not unexpected in view of the virtual absence of conjugation between the thioxanthone nucleus and the amine side chain, concomitant with protonation of N-11.

It is not uncommon for steric inhibition of resonance to be associated with the appearance of an absorption band which is not seen in the spectrum of the fully conjugated compound. Examples include sterically hindered

derivatives of biphenyl (12, 24), and 1,2′-acetylvinylcyclohexene (25, 26). In the former case a possible explanation, based on a consideration of localized self-consistent field orbitals, has been adduced by Murrell (24). In the second example, the band is thought to correspond to an electronic transition in the enone side chain. In such cases it is postulated that conjugation is reduced to such a degree that the parts of the molecule linked by the distorted bond act as separate chromophores. In general, such bands need not represent added electronic transitions. Rather, they may indicate a separation of distinct electronic excitations which comprise a single, unresolved band complex in the spectrum of the unhindered compound. This concept may also serve to explain the presence of the shoulders in the low-frequency portions of the 335 and 253 nm band envelopes of N^{11}-methyllucanthone.

It should be noted that the effects of N^{11}-methyl substitution may be due in part to loss of an intramolecular (N—H···O) hydrogen bond. This applies particularly to the frequency shifts. However, the composite spectral changes are fully consistent with steric inhibition of resonance. (The presentation and discussion of findings pertinent to the possible presence of an intramolecular hydrogen bond in lucanthone are deferred to a subsequent report.)

Ionization Constants

When hydrogen chloride is added to ethereal solutions of the free diamines, stable hydrochlorides are obtained. Quantitative analysis indicates that the salt of lucanthone is a *mono-* and that of its N^{11}-methyl derivative a *di*hydrochloride (4, 5). The infrared spectrum of the stable hydrochloride of lucanthone (Fig. 3) manifests the N—H stretching vibration (3250 cm^{-1}) in the presence of the typical ammonium band of a tertiary amine (2650–2490 cm^{-1}): it is clearly separated from the various C—H stretching frequencies at 3060–2870 cm^{-1} (27). It may be inferred that only the terminal, tertiary amine is protonated. The findings are consistent with an increase in the basicity of the proximal amine substituent concomitant with N^{11}-methyl substitution. The quantitative definition of this difference is described below.

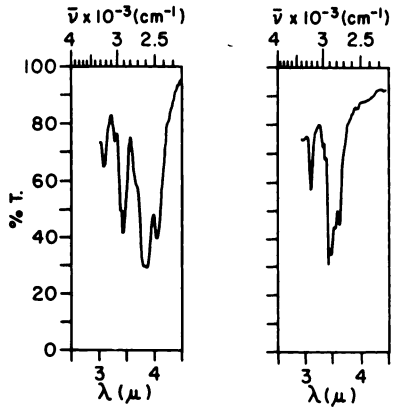

FIG. 3. *Infrared spectra of the hydrochloride (left) and free diamine of lucanthone (KBr discs)*

λ = wavelength; ν̄ = wave number; T = transmittance. Note the persistence of the N—H stretching band at 3.08 μ (3250 cm^{-1}) in the spectrum of the stable hydrochloride. This clearly indicates that only the terminal, tertiary amine substituent is protonated (see the text).

Ionization constants of the two thioxanthones were determined by spectrophotometric methods. Spectra were determined over a wide range of acidity, extending at least 4.3 H_0 units below and 5.7 pH units above the estimated pK_a of the N-11 amine group. Aside from a distinct medium effect (see below) in strongly acid solutions, no spectral changes occurred other than those attributable to changes in the degree of ionization of a single substituent. The attainment of precise, monophasic titration curves indicates the absence of extraneous influences and attests to the validity of the methods employed.

Lucanthone. Analysis of recorded spectra revealed a definite medium effect: as the acidity of the solvent increased, the absorption maxima manifested progressive bathochromic shifts without significant changes in bandwidth or intensity. The findings are identical with those described by other investigators (6, 7). Shifts due to the medium effect preclude the existence of true isosbestic points and result in a false relationship between the absorptivity of a base and its conjugate acid at any given wavelength. Satisfactory correction of the results was

obtained by solving each set of equations by the method of least squares, as suggested by Flexser, Hammett, and Dingwall (6).

Two sets of five determinations each were obtained at the absorption maximum of the dihydrochloride, and one set of five determinations, at the maximum of the monohydrochloride. The mean of all 15 determinations was $pK_a = -0.20$, with a *maximum* deviation of ± 0.05 and an average deviation of ± 0.02.

N^{11}-Methyllucanthone. The mean pK_a, based on 15 determinations at the absorption maximum of the dihydrochloride, was 3.41, with a *maximum* deviation of ± 0.04 and an average deviation of ± 0.02.

Differences in the chloride composition of the stable salts of the two thioxanthones were first noted by Elslager, Cavalla, Closson, and Worth (5), who attributed the lower basicity of the secondary amine of lucanthone to hydrogen bonding (*sic*). In this regard, the data of Peters and Sumner (28) are pertinent. These investigators determined the influence of N—H···O hydrogen bonds on the basic ionization constants (K_b) of a series of amino, methylamino, and dimethylamino anthraquinones. Their results indicate that intramolecular hydrogen bonds affect K_b by a factor of 10 or less. By contrast, the basic ionization constant of 1-dimethylaminoanthraquinone, in which the steric configuration is virtually identical with that of N^{11}-methyllucanthone, manifests a 1000-fold increase over that of unhindered derivatives. Consequently, the magnitude of the difference between the ionization constants of the two thioxanthones implies that steric inhibition of resonance is the dominant factor in effecting the enhanced basicity of the N^{11}-methyl derivative.

DISCUSSION

The present study suggests several hypotheses which may serve as bases for further investigation. (a) Coplanarity of the thioxanthone ring and amine side chain may be required for optimum biological activity. (b) Changes in physical properties concomitant with steric inhibition of resonance may account for the observed differences in cytotoxic and biochemical effects; this possibility is currently under investigation.

An alternative hypothesis has been proposed by Blanz and French (2), who suggest that diminished hydrogen-bonding capacity may be responsible for the reduced carcinostatic activity of the N^{11}-methyl derivatives. Studies on this aspect of the problem have been completed and will be reported in a separate communication.

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REFERENCES

1. E. Hirschberg, I. B. Weinstein, N. Gersten, E. Marner, T. Finkelstein and R. Carchman, *Cancer Res.* **28**, 601 (1968).
2. E. J. Blanz and F. A. French, *J. Med. Chem.* **6**, 185 (1963).
3. R. A. Carchman, E. Hirschberg and I. B. Weinstein, *Biochim. Biophys. Acta* **179**, 158 (1969).
4. H. Mauss, *Chem. Ber.* **81**, 19 (1948).
5. E. F. Elslager, J. F. Cavalla, W. D. Closson and D. F. Worth, *J. Org. Chem.* **26**, 2837 (1961).
6. L. A. Flexser, L. P. Hammett and A. Dingwall, *J. Amer. Chem. Soc.* **57**, 2103 (1935).
7. M. A. Paul and F. A. Long, *Chem. Rev.* **57**, 1 (1957).
8. A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," pp. 12, 81. Methuen, London, 1962.
9. L. P. Hammett and A. J. Deyrup, *J. Amer. Chem. Soc.* **54**, 2721 (1932).
10. A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," p. 69. Methuen, London, 1962.
11. C. A. Coulson, *in* "Steric Effects in Conjugated Systems" (G. W. Gray, ed.), p. 8. Butterworths, London, 1958.
12. G. H. Beaven, *in* "Steric Effects in Conjugated Systems" (G. W. Gray, ed.), p. 22. Butterworths, London, 1958.
13. C. C. Barker, *in* "Steric Effects in Conjugated Systems" (G. W. Gray, ed.), p. 34. Butterworths, London, 1958.
14. M. J. S. Dewar, *in* "Steric Effects in Con-

jugated Systems" (G. W. Gray, ed.), p. 46. Butterworths, London, 1958.
15. W. F. Forbes, *in* "Steric Effects in Conjugated Systems" (G. W. Gray, ed.), p. 62. Butterworths, London, 1958.
16. E. S. Waight and R. L. Erskine, *in* "Steric Effects in Conjugated Systems" (G. W. Gray, ed.), p. 73. Butterworths, London, 1958.
17. B. M. Wepster, *in* "Steric Effects in Conjugated Systems" (G. W. Gray, ed.), p. 82. Butterworths, London, 1958.
18. H. H. Jaffe and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," p. 389. Wiley, New York, 1962.
19. H. Suzuki, "Electronic Spectra and Geometry of Organic Molecules," pp. 261, 445, 468, 485, 496. Academic Press, New York, 1967.
20. H. Suzuki, "Electronic Spectra and Geometry of Organic Molecules," pp. 59, 342. Academic Press, New York, 1967.
21. H. Suzuki, *Bull. Chem. Soc. Jap.* **35**, 1715 (1962).
22. H. H. Jaffe and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," pp. 137, 392. Wiley, New York, 1962.
23. J. Dale, *in* "Steric Effects in Conjugated Systems" (G. W. Gray, ed.), p. 72. Butterworths, London, 1958.
24. J. N. Murrell, *J. Chem. Soc.* 3779 (1956).
25. E. A. Braude, E. R. H. Jones, H. P. Koch, R. W. Richardson, F. Sondheimer and J. B. Toogood, *J. Chem. Soc.* 1890 (1949).
26. E. A. Braude and E. R. H. Jones, *J. Amer. Chem. Soc.* **72**, 1041 (1950).
27. K. Nakanishi, T. Goto and M. Ohashi, *Bull. Chem. Soc. Jap.* **30**, 403 (1957).
28. R. H. Peters and H. H. Sumner, *J. Chem. Soc.* 2101 (1953).