50 μg of II/ml, showed averages of actual content measured in 36 samples to be 98.7 ± 4.2% (I) and 97.1 ± 4.1% (II).

Following intravenous administration of 10 mg of I/kg to a dog, serum levels of I and II, measured by this HPLC method and a GLC method (6), were compared (Fig. 3). The two methods differed by an average of 9% in the measurement of total drug-related material (I plus II) in the nine serum samples compared.

REFERENCES

(1) M. Nemati, M. C. Kyle, and E. D. Freis, *J. Am. Med. Assoc.*, **237**, 652 (1977).

(2) J. G. R. de Carvalho, F. G. Dunn, S. G. Chrysant, and E. D. Frohlich, *Arch. Intern. Med.*, **138**, 53 (1978).

(3) J. C. Levron, J. M. LeFur, P. Adnot, and Y. Dormard, *Eur. J. Drug Metab. Pharmacokinet.*, **2**, 107 (1977).

(4) G. Maurer, M. Roche, P. Darmenton, and H. Pacheco, *ibid.*, **1**, 202 (1976).

(5) J. P. Desager, M. Vanderbist, B. Hwang, and P. Levandoski, *J. Chromatogr.*, **123**, 379 (1976).

(6) B. Hwang, G. Konicki, R. Dewey, and C. Miao, *J. Pharm. Sci.*, **67**, 1095 (1978).

ACKNOWLEDGMENTS

The authors thank Dr. Bruce Hwang and Mr. George Konicki for performing GLC analyses with which the HPLC method was compared and Ms. Janice Christie for typing the manuscript.

NOTES

Frozen Conformers of Clotrimazole: Reaction of Imidazole with 1-Chloro-9-hydroxy-9-phenylxanthene

PAUL L. WARNER, Jr.ˣ, EDWARD LUBER, Jr., and F. CHRISTOPHER ZUSI

Received March 1, 1979, from the *Department of Medicinal Chemistry, Westwood Pharmaceuticals Inc., Buffalo, NY 14213.* Accepted for publication May 3, 1979.

Abstract □ 1-Chloro-9-hydroxy-9-phenylxanthene reacts with imidazole at 180° to form a 5:1 mixture of the 9-(imidazo-1-yl)- and 9-(imidazo-2-yl)-1-chloro-9-phenylxanthenes. These products lack significant antifungal activity.

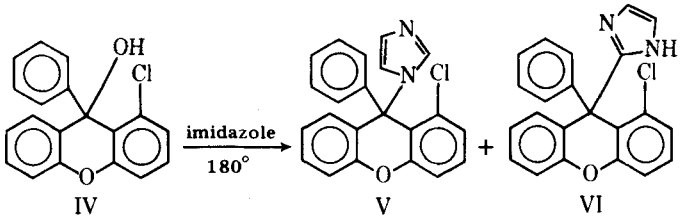
Keyphrases □ Clotrimazole—analogs, frozen conformers, imidazole substitution, antifungal activity □ Antifungal agents—clotrimazole analogs, frozen conformers, imidazole substitution, antifungal activity

The advent of clotrimazole (I) (1, 2) as a clinically useful, broad spectrum antifungal agent has elicited considerable research on the synthesis and testing of analogs of this molecule including the preparation of "frozen conformers." The fluorene analogs II and III were prepared and exhibited good antifungal activity (3).

DISCUSSION

Various other compounds in which the covalent bridge between two phenyl rings (as in II and III) is replaced by sulfur, oxygen, ethylene, or polymethylene have been reported (4–7). The usual synthetic method for these compounds has been displacement of the appropriate tertiary carbinol with thionyl bis-*N,N'*-imidazole (4, 5) or displacement of the appropriate tertiary chloride with imidazole by refluxing in benzene (3) or acetonitrile (6, 7). In all cases, the expected N_1-imidazole derivative was obtained with no mention of other isomers.

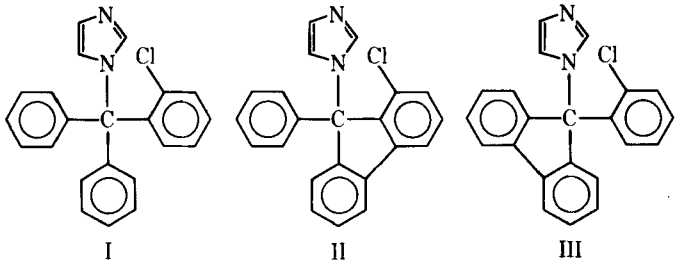
To elucidate the pharmacophoric conformation of clotrimazole, 1-chloro-9-(imidazo-1-yl)-9-phenylxanthene (V) was prepared. When 1-chloro-9-hydroxy-9-phenylxanthene (IV) was fused with imidazole at 180°, the expected V was isolated in a 46% yield along with a 9% yield of the unexpected 1-chloro-9-(imidazo-2-yl)-9-phenylxanthene (VI) (Scheme I).



Scheme I

Structures V and VI were assigned to the two reaction products based on their identical elemental analysis and their physical and spectral properties. Compound V displayed considerably greater mobility on silica gel in accord with its expected lower basicity. The IR spectrum of VI contained an absorption at 2850 cm^{-1} corresponding to the imidazole NH bond, which was absent from V. In the 100-MHz NMR spectrum, both V and VI showed a complex aromatic splitting pattern from δ 6.8 to 7.4. Compound V displayed a pair of overlapping triplets at δ 7.5 and 7.6 corresponding to two imidazole ring CH protons, each individually coupled to the two ring protons (the third proton apparently was concealed under the major aromatic peaks). Compound VI showed two distorted doublets at δ 6.4 and 7.55, each a single proton. These doublets sharpened on deuterium oxide exchange, indicating that a free NH was present but also concealed under the main aromatic peak.

Neither V nor VI displayed significant antifungal activity compared to clotrimazole (Table I).



EXPERIMENTAL[1]

Chemistry—*1-Chloro-9-hydroxy-9-phenylxanthene (IV)*—To a stirred suspension of 4.4 g (0.019 mole) of 1-chloroxanthone (8) in 100 ml of anhydrous ether was added dropwise, over 10 min, 22 ml (0.039

[1] NMR spectra were obtained on a Varian XL-100. IR spectra were taken on a Perkin-Elmer model 281 spectrophotometer. UV spectra were taken in absolute ethanol on a Beckman DB-G grating spectrophotometer. Elemental analyses were obtained from Galbraith Laboratories, Knoxville, Tenn. Melting points were taken on a Mel-Temp melting-point apparatus and are uncorrected.

0022-3549/79/1100-1453$01.00/0

Table I—Minimum Inhibitory Concentration [a] by Agar Dilution Method in Sabouraud's Dextrose Agar

Test Organism	V[b]	VI[c]	Clotrimazole
Candida albicans (two strains)	>256	>512	1
Candida tropicalis	>256	>512	1
Rhodotorula sp.	>256	>512	8
Aspergillus niger	>256	>512	8
Trichophyton mentagrophytes	>256	>512	0.5

[a] Micrograms per milliliter of medium. [b] Target concentration was 256 μg/ml. [c] Target concentration was 512 μg/ml.

mole) of 1.76 *M* phenyllithium in benzene–ether[2]. After stirring at room temperature for 15 min, the reaction was gently refluxed with stirring for 1 hr. The cooled mixture was treated carefully dropwise with 10 ml of saturated aqueous ammonium chloride followed by 20 ml of water. The organic layer was separated, washed with water, dried with anhydrous magnesium sulfate, and evaporated.

The residual oil was then triturated with petroleum ether and filtered, and the solid was crystallized from an ethanol–hexane mixture to provide 4.3 g (73.4%) of white crystals, mp 131–134.5°; UV: λ_{max} 236 (A_m = 13,100), 281 (3000), and 290 (4400) nm; IR (KBr): 3540, 3450, 3065, 1595, 1570, 1445, 1115, and 755 cm^{-1}.

Anal.—Calc. for $C_{19}H_{13}ClO_2$: C, 73.91; H, 4.24; Cl, 11.48. Found: C, 74.45; H, 4.27; Cl, 11.54.

1-Chloro-9-(imidazo-1-yl)-9-phenylxanthene (V) and 1-Chloro-9-(imidazo-2-yl)-9-phenylxanthene (VI)—A mixture of 0.9 g (0.003 mole) of IV and 3.0 g (0.44 mole) of imidazole was heated under a nitrogen atmosphere at 180° for 5 hr. After cooling, the reaction mixture was digested with 30 ml of water, and the insoluble solid was separated by filtration. The solid was dissolved in chloroform, chromatographed on 75 g of a 2-cm silica gel (40–140 mesh) column[3], eluted with ether–chloroform (1:1), and collected in 100-ml fractions. Compound V eluted first followed by VI. Following evaporation of the appropriate fractions, both V and VI were crystallized from ethanol–water.

For V, the yield was 0.5 g (46%), mp 235–237°; R_f 0.7 [silica gel GF, ether–chloroform (1:1 v/v)]; UV: λ_{max} 285 (A_m = 3280) nm; IR (KBr): 3065, 1590, 1570, 1440, 1368 (CN), 1110, 1100, and 755 cm^{-1}; NMR ($CDCl_3$–dimethyl sulfoxide-d_6): δ 6.8–7.4 (m, 13H, aromatic) and 7.5 and 7.6 (overlapping triplet, 1H each, imidazole ring H).

Anal.—Calc. for $C_{22}H_{15}ClN_2O$: C, 73.64; H, 4.21; Cl, 9.88; N, 7.81. Found: C, 73.89; H, 4.31; Cl, 10.00; N, 7.76.

[2] Aldrich Chemical Co.
[3] Baker.

For VI, the yield was 0.1 g (9%), mp 223–225°; R_f 0.3 (same system as for V); UV: λ_{max} 285 (A_m = 3140) nm; IR (KBr): 3070, 2860 (imidazole NH), 1590, 1570, 1440, 1100, 1090, and 755 cm^{-1}; NMR ($CDCl_3$–dimethyl sulfoxide-d_6): δ 6.48 (distorted doublet, 1H, imidazole CH), 6.8–7.4 (m, 13H, aromatic H), and 7.55 (distorted doublet, 1H, imidazole CH); NMR (dimethyl sulfoxide-d_6–D_2O): δ 6.33 (d, imidazole CH), 6.8–7.4 (m, aromatic H), and 7.54 (d, imidazole CH).

Anal.—Calc. for $C_{22}H_{15}ClN_2O$: C, 73.64; H, 4.21; Cl, 9.88; N, 7.81. Found: C, 73.61; H, 4.40; Cl, 9.91; N, 7.66.

Mycology—The antifungal activity of V and VI, using clotrimazole as a reference, was determined by using a Steer's replicator (9) to inoculate plates of Sabouraud's dextrose agar containing twofold serial dilutions of the compounds. The replicator wells contained fungal suspensions at concentrations greater than 6×10^5 colony-forming units/ml. The minimum inhibitory concentration was determined as the lowest concentration that prevented substantial growth of the test organisms. The data summarized in Table I show that neither V nor VI was active against fungi at the concentrations tested.

REFERENCES

(1) K. H. Buchel, E. Regel, and M. Plempel, U.S. pat. 3,660,577 (1972).

(2) P. R. Sawyer, R. N. Brogden, R. M. Pinder, T. M. Speight, and G. S. Avery, *Drugs*, **9**, 424 (1975).

(3) S. Casadio, A. Donetti, and G. Coppi, *J. Pharm. Sci.*, **62**, 773 (1973).

(4) W. Draber, H. Timmler, K. H. Buchel, and M. Plempel, U.S. pat. 3,814,802 (1974).

(5) *Ibid.*, U.S. pat. 3,778,447 (1973).

(6) W. Draber, K. H. Buchel, and M. Plempel, U.S. pat. 3,737,548 (1973).

(7) *Ibid.*, U.S. pat. 3,647,816 (1972).

(8) A. A. Goldberg and A. H. Wragge, *J. Chem. Soc.*, **1958**, 4234.

(9) E. Steers, E. L. Foltz, and B. S. Graves, *Antibiot. Chemother.*, **9**, 307 (1959).

ACKNOWLEDGMENTS

The authors thank the Analytical Chemistry Research Department, Bristol Laboratories, Syracuse, N.Y., for the NMR spectra and the Westwood Microbiology Department for the antifungal testing data. They also thank Delbay Pharmaceuticals, Inc., Bloomfield, N.J., for a clotrimazole sample and Dr. A. J. Solo, State University of New York at Buffalo, for reviewing the manuscript.

High-Performance Liquid Chromatographic Determination of (*Z*)- and (*E*)-Doxepin Hydrochloride Isomers

T. J. WHALL ˣ and J. DOKLADALOVA

Received February 15, 1979, from the *Special Testing and Analytical Development Laboratories, Quality Control Division, Pfizer Inc., Groton, CT 06340.* Accepted for publication May 8, 1979.

Abstract □ A high-performance liquid chromatographic method for the determination of (*Z*)- and (*E*)-doxepin hydrochloride isomers was developed. The analysis employs a column packed with spherical silica microparticles (5–6 μm), and a mobile phase of acetonitrile–chloroform–diethylamine (750:250:0.2) permits baseline resolution and simultaneous determination of the (*Z*)- and (*E*)-doxepin isomers. Process-related substances do not interfere. The method is accurate and precise (the relative standard deviation was 0.3% for both isomers). The simple procedure is highly suitable for routine doxepin hydrochloride analysis.

Keyphrases □ Doxepin hydrochloride—isomers, high-performance liquid chromatographic analysis □ Antidepressants—doxepin hydrochloride, isomers, high-performance liquid chromatographic analysis □ High-performance liquid chromatography—analysis, doxepin hydrochloride isomers

Doxepin hydrochloride, 3-dibenz[*b,e*]oxepin-11(6*H*)-ylidene-*N,N*-dimethyl-1-propanamine hydrochloride, is a dual-action antianxiety and antidepression psychotherapeutic agent (1). Chemically, it is a mixture of (*Z*)- and (*E*)-isomers (I*a* and I*b*, respectively) in an ~15:85% ratio. Recently, a GLC procedure (2), which requires an extraction step to convert the hydrochloride to free base, was recommended for doxepin hydrochloride analysis.

0022-3549/79/1100-1454$01.00/0