dose and raised the minimum effective dose; so did two CH_3O groups at the 2- and 5-positions on the benzylidene groups.

The low toxicity of the indene derivatives might have been predicted on the basis of the relatively high ED_{50} figures found in KB cell culture tests, but the antitumor activity would not have been. It is interesting to note that some alkoxybenzylidene derivatives of indene, without any amino group, were among the most active compounds in inhibiting cell culture growth.

The ultraviolet absorption spectra of the aminobenzylideneindenes and cyclopentadienes showed peaks in the 360–420-mμ region, usually near 400 mμ, in methanol, as did the styrylquinolines, but in most cases the peaks were reduced in wave length or intensity or both by acetic acid. The compounds without amino groups had about the same maxima in both solvents.

Experimental

In a typical preparation, a solution of 0.04 mole of *p*-dialkylaminobenzaldehyde and 0.045 mole of indene in 200 ml. of absolute ethanol was heated to boiling, then 50 ml. of a saturated solution of KOH in absolute ethanol was added and the mixture was refluxed 25 min. The product which crystallized on chilling the solution was recrystallized from acetone or other convenient solvent. In some instances it was necessary to add water and precipitate the product as an oil. When cyclopentadiene was used in place of indene, the reaction mixture was prepared at room temperature and allowed to stand 30 min. before heating.

Nuclear Magnetic Resonance Spectra of Phenothiazines. Chemical Shift Data[1]

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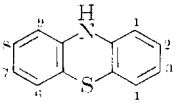
The widespread use of phenothiazine tranquilizers has given rise to extensive investigations into the structure of their metabolites,[3] variously thought to be hydroxylated in the 3-, 7-,[4] or 8-positions.[5] Since the infrared spectrum will not readily distinguish, *e.g.*, a 2,7-disubstituted phenothiazine from its 2,8-isomer[6] (both being 1,2,4-trisubstituted benzenes), and since the necessary synthetic hydroxylated phenothiazines are not yet available, the use of n.m.r. spectroscopy offered an attractive solution to the problem of unambiguous structure assignment of the metabolites of the phenothiazine drugs, if individual substitution sites in this molecule could be distinguished. This has now been shown to be feasible by an analysis of the n.m.r. spectra of the four possible monosubstituted compounds and of three types of disubstituted phenothiazines bearing a substituent in each benzene ring in the 2,7-, 2,8-, or 3,7-positions. In each case one substituent was a chloro or methoxy group. The preparation and n.m.r. spectra of 1,4- and 2,3-disubstituted compounds will be described in another paper.

Experimental

N.m.r. spectra were recorded using a Varian A-60 spectrometer. Chemical shifts are given in c.p.s. downfield from an internal tetramethylsilane standard. Approximately 5–10% solutions of the phenothiazines in perdeuteriodimethyl sulfoxide were prepared in a drybox under nitrogen. Dimethyl sulfoxide was found to inhibit autoxidation of the compounds. Melting points were taken in sealed evacuated capillary tubes on a Thomas–Hoover melting point apparatus, and are corrected. This method was found to give sharp melting points, frequently higher than those quoted in the literature, by suppressing the oxidation which inevitably occurred on using either a Kofler hot stage or an open capillary tube. Compounds were rigorously purified by repeated vacuum sublimation and were stored in sealed ampoules under nitrogen. A list of the substances[7] examined is given in Table I.

TABLE I

PHENOTHIAZINES USED TO OBTAIN CHEMICAL SHIFTS



Substitution at					
C-1	C-2	C-3	C-7	C-8	M.p., °C.
OMe					100.2–101.0
	OMe				184.0–184.5
		OMe			166.5–167.5
Cl					96.2–96.7
	Cl				202.8–203.2
		Cl			203–204
	CF_3		OMe		169–170
	CF_3			OMe	138.5–140
	CF_3			Cl	188.5–189
		OMe	OMe		201.0–202.5
	Cl		OMe		174.0–174.8
	Cl			Cl	269.5–270.5
	Cl		Cl		214.5–215.0
		Cl	Cl		239.5–240.0
Phenothiazine					184.0–184.5
10-Methylphenothiazine					102.5–103.0

Results

Comparison of the n.m.r. spectra of phenothiazine and 2-chlorophenothiazine on one hand, and those of the corresponding 10-(3-dimethylaminopropyl)substituted phenothiazines (promazine and chlorpromazine) on the other, showed clearly that the only difference between the spectra of each N-unsubstituted and N-substituted pair was the *bulk* chemical shift of all common hydrogen atoms. The individual chemical shifts of each aromatic hydrogen atom, and the coupling constants, were the same.

Analysis of the chemical shift data for phenothiazine and chlorine-substituted phenothiazines (in which the hydrogen frequencies are not altered by shielding[8])

(1) This investigation was supported by the National Institute of Mental Health, U. S. Public Health Service, through Research Grant MH-4582 and Contract PH 43-63-53.

(2) To whom inquiries should be addressed.

(3) For review, see J. L. Emerson and T. S. Miya, *J. Pharm. Sci.*, **52**, 411 (1963).

(4) H. Goldenberg and V. Fishman, *Proc. Soc. Exptl. Biol. Med.*, **108**, 178 (1961).

(5) I. S. Posner, R. Culpan, and J. Levine, *Federation Proc.*, **22**, 540 (1963).

(6) J. C. Craig, W. P. Rogers, and M. E. Tate, *Australian J. Chem.*, **9**, 397 (1956).

(7) We thank Dr. E. Jucker (Sandoz A. G., Basel) for a sample of 2-methoxyphenothiazine and Dr. P. N. Craig (Smith Kline and French Laboratories, Philadelphia, Pa.) for samples of 1-chlorophenothiazine and the 2-trifluoromethyl compounds listed in Table I.

(8) L. M. Jackman, "Applications of Nuclear Magnetic Resonance in Organic Chemistry," Pergamon Press, Oxford, 1959.

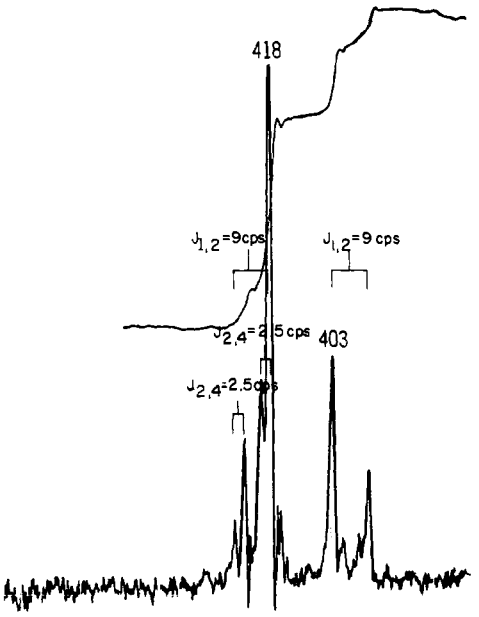


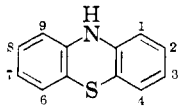
Figure 1.—N.m.r. spectrum (60 Mc.) of 3,7-dichlorophenothiazine in perdeuteriodimethyl sulfoxide. Field increases from left to right. Chemical shifts are in c.p.s. downfield from an internal tetramethylsilane reference.

gave the values summarized in Table II. The strong interaction between neighboring hydrogens (spin–spin coupling) which exists between *ortho* hydrogens (J = 5 to 10 c.p.s.), and the weaker couplings by *meta* hydrogens (J = 1 to 5 c.p.s.), are very sensitive to substitution. When substitution takes place in the 3-position, the 3,4-*ortho* coupling disappears and only the large 1,2-, 6,7-, and 8,9-*ortho* couplings remain. Disubstitution at the 2,8-positions leaves only the 3,4- and 6,7-*ortho* couplings and weak *meta* coupling of H-1 and H-9. Disubstitution at 3,7 leaves only 1,2- and 8,9-*ortho* couplings and weak *meta* coupling of H-4 and H-6. A combination of chemical shift, spin–spin couplings, and integration data permits the identification of individual hydrogens at each site in the aromatic rings.

The general features of the n.m.r. spectra are illustrated by the typical curves reproduced in Figures 1–4, which show the effect of substitution on the resonance frequencies of the aromatic hydrogens in these compounds.

TABLE II

CHEMICAL SHIFTS OF AROMATIC HYDROGENS IN CHLORO-SUBSTITUTED PHENOTHIAZINES



Hydrogen	Chemical shifts,[a] c.p.s.	Coupling constants, c.p.s.
1	395(m)–401(o)	$J_{1,2}$ = 9
2	423(o)	
3	404(o)	
4	414(m)–418(o)	
6	419(m)–420(o)	$J_{6,8}$ = 2
7	405(o)	
8	422(o)	
9	396(m)–401(o)	$J_{8,9}$ = 8

[a] Shifts are listed for hydrogens substituted either *ortho* (o) or *meta* (m) to a chlorine atom.

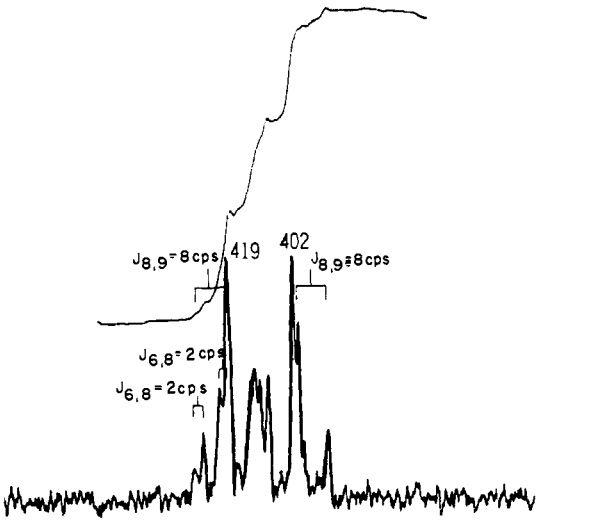


Figure 2.—N.m.r. spectrum (60 Mc.) of 2,7-dichlorophenothiazine in perdeuteriodimethyl sulfoxide. Field increases from left to right. Chemical shifts are in c.p.s. downfield from an internal tetramethylsilane reference.

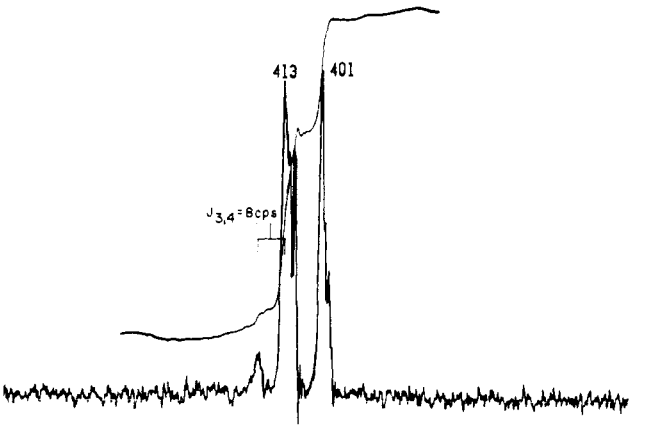


Figure 3.—N.m.r. spectrum (60 Mc.) of 2,8-dichlorophenothiazine in perdeuteriodimethyl sulfoxide. Field increases from left to right. Chemical shifts are in c.p.s. downfield from an internal tetramethylsilane reference.

In 3,7-dichlorophenothiazine (Figure 1) the doublet centered at 395 c.p.s. is one-half of an AB system belonging to the C-1 hydrogen with $J_{1,2}$ = 9 c.p.s. The other half of the AB due to the C-2 hydrogen at 423 c.p.s. is further split by the *meta* hydrogen on C-4 with $J_{2,4}$ = 2.5 c.p.s. The tall sharp line at 418 c.p.s. is a superposition of the C-4 hydrogen and part of the AB coupling from the C-3 hydrogen. The ratio of the integrated intensities of the two groups of hydrogens was 2:1.

The spectrum of 2,7-dichlorophenothiazine (Figure 2) shows two peaks centered about 396 c.p.s. which are due to part of the AB coupling between hydrogens at C-8 and C-9 and are assigned to the C-9 hydrogen ($J_{8,9}$ = 8 c.p.s.). The adjacent line at 402 c.p.s. is caused by the hydrogen on C-1. The low-field doublet at 421 c.p.s. is the other half of the AB system, arising from the C-8 hydrogen, and is further split by *meta* coupling with the C-6 hydrogen ($J_{6,8}$ = 2 c.p.s.). The line at 419 c.p.s. is assigned to the hydrogen on C-6, while the multiplet centered about 412 c.p.s. is part of an AB system involving the C-3 and C-4 hydrogens, the re-

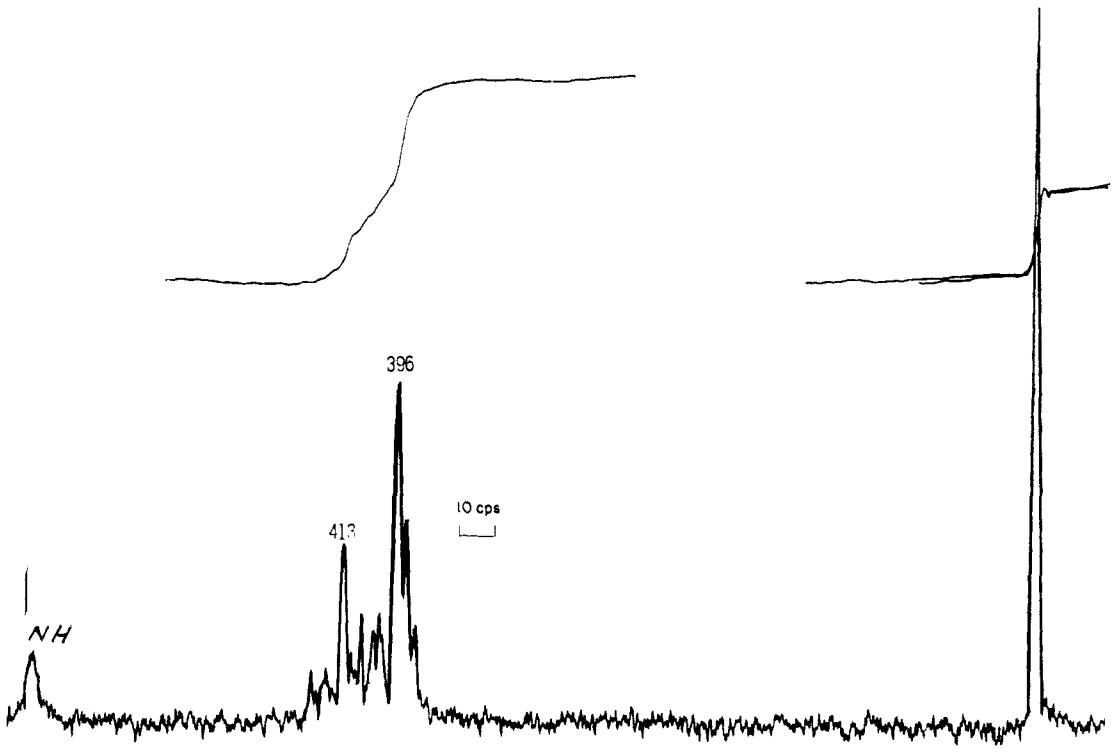


Figure 4.—N.m.r. spectrum (60 Mc.) of 3-methoxyphenothiazine in perdeuteriodimethyl sulfoxide. Field increases from left to right. Chemical shifts are in c.p.s. downfield from an internal tetramethylsilane reference.

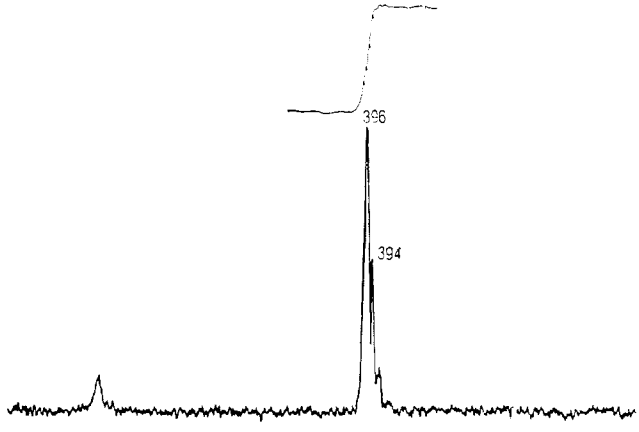


Figure 5.—N.m.r. spectrum (60 Mc.) of 3,7-dimethoxyphenothiazine in perdeuteriodimethyl sulfoxide. Field increases from left to right. Chemical shifts are in c.p.s. downfield from an internal tetramethylsilane reference.

maining half of which lies under the peaks assigned to hydrogens at C-6 and C-1. Integration gave a 1:1:1 ratio, as expected.

The assignments for 3,7- and 2,7-dichlorophenothiazine may be further verified by comparing the overlapping peaks of the two spectra. The peaks at 407–413 c.p.s. in 2,7-dichlorophenothiazine are absent in the 3,7-dichloro compound, as is the sharp peak at 400 c.p.s. Since one ring in these two substances is the same, the resonance lines should be identical, provided that there is no interaction across the thiazine ring. Therefore, the peaks which are present only in the 2,7-dichlorophenothiazine spectrum must belong to the hydrogens at C-3 and C-4 as suggested above.

In 2,8-dichlorophenothiazine (Figure 3) both aromatic rings are identical. The doublet centered about 418 c.p.s. is part of an AB system due to the C-3 and C-4 hydrogens, and is assigned to the hydrogen at C-4, since no *meta* splitting is observed. The multiplet at 400 c.p.s. is assigned to the C-1 hydrogen with a small *meta* coupling due to the C-3 hydrogen. This group also includes part of the AB system of the hydrogen at C-3. The signal at 408 c.p.s. contains part of the AB coupling of hydrogens at C-3 and C-4.

The spectrum of 3-methoxyphenothiazine (Figure 4) demonstrates the strong shielding caused by the methoxy group. There the C-1, C-2, and C-4 hydrogens are clustered together at 396 c.p.s. There appears to be an ABX pattern for the hydrogens at C-6 and -7 and C-8 and -9, in which the two sets are equivalent, one portion of the AB (at 415 c.p.s.) being due to hydrogens at C-7 and C-8, and the other (407 c.p.s.) attributed to those at C-6 and C-9, as verified by the ratio of the integrated intensities (1:1).

Both 3,7-dimethoxy- and 2-chloro-7-methoxyphenothiazine (Figures 5 and 6) showed the same effect, reducing the chemical shift between hydrogens at positions 6, 8, and 9, and resulting in an incompletely resolved multiplet at 396 c.p.s.

In the last compound, the doublet centered at 415 c.p.s. is part of the AB system of the C-3 and C-4 hydrogens, and is assigned to that at C-4. A small doublet at 405 c.p.s. represents the remaining half of the AB coupling, due to hydrogen at C-3, and also shows *meta* coupling by the C-1 hydrogen, which is present in the multiplet at 396 c.p.s. Integrated intensities were in agreement with the predicted ratio of 1:1.8.

A comparison of 2,8-dichloro- and 3,7-dimethoxyphenothiazine shows that the signals due to hydrogens at C-6, -8, and -9 must be located at 396 c.p.s., and that the first two peaks centered at 418 c.p.s. must be part of the AB system of the C-4 hydrogen.

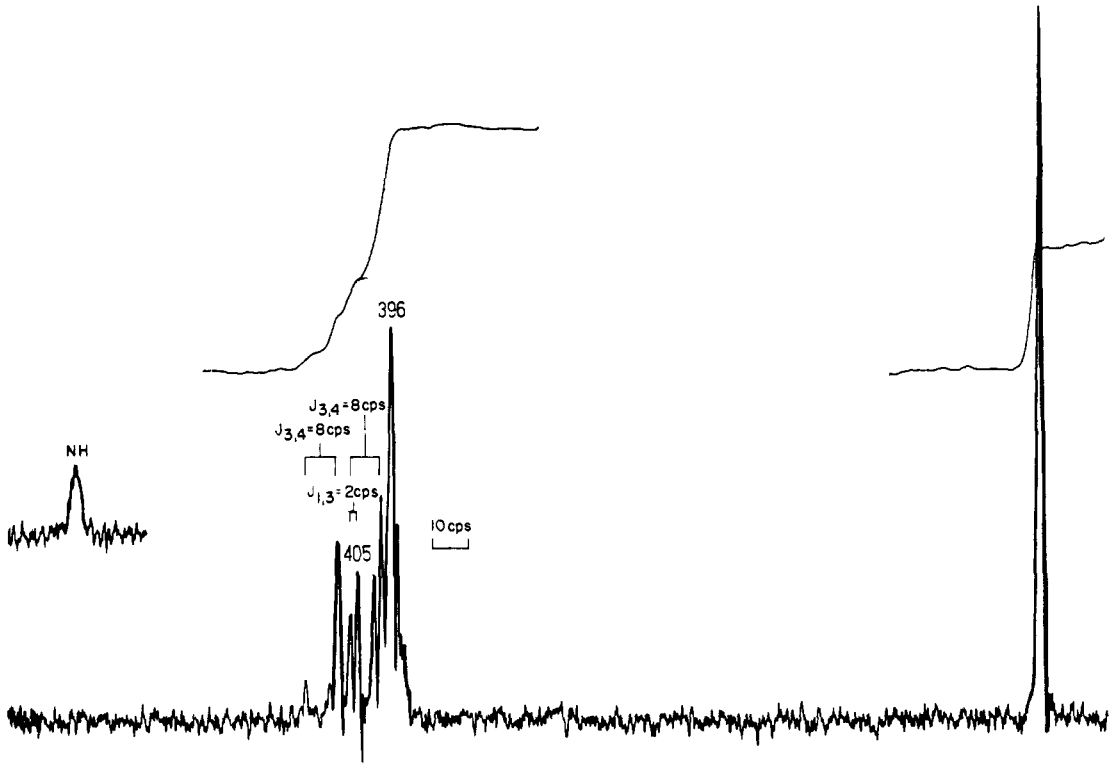


Figure 6.—N.m.r. spectrum (60 Mc.) of 2-chloro-7-methoxyphenothiazine in perdeuteriodimethyl sulfoxide. Field increases from left to right. Chemical shifts are in c.p.s. downfield from an internal tetramethylsilane reference.

The Synthesis, Proof of Structure, and Biological Activity of Some Monosubstituted Aminoguanidines[1]

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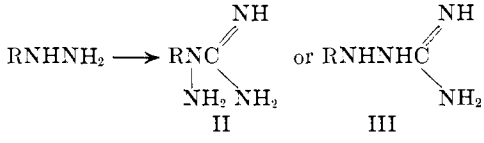
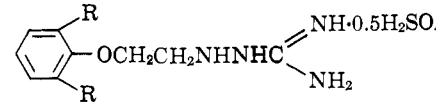
Received November 2, 1964

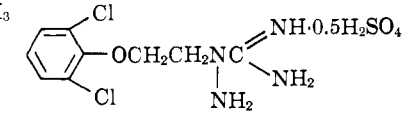
The reaction of monosubstituted hydrazines with S-methylisothiourea sulfate (I) has been claimed[2] to yield substituted aminoguanidines of type II. We have found that 2-(2,6-disubstituted phenoxy)ethylhydrazines react with I to give, as the main product, aminoguanidines of type III.



Reaction of 2-(2,6-dichlorophenoxy)ethylhydrazine with I yielded a compound which was assigned structure IVa,[3] on the basis of its failure to give a benzal derivative. As this compound (guanoclor[4]) displayed both dopamine β-oxidase inhibitory and antihypertensive properties,[5] it was of interest to synthesize the isomer



IVa, R = Cl
b, R = CH_3



V

V and examine its biological properties. Comparison of this compound with IVa and the analog IVb, the latter two synthesized by equivocal guanylation of the appropriate hydrazine,[3] confirmed the assignment of the structure IV on the following grounds.

(i) Derivatives.—In contrast to V, which readily gave a benzal derivative, IVa was recovered unchanged after prolonged treatment with benzaldehyde.

(ii) Degradation.—Treatment of IVb with Raney nickel gave 2-(2,6-dimethylphenoxy)ethylamine and guanidine sulfate. Similar treatment of IVa and V did not yield pure products due to partial loss of chlorine. However, in the former case, some guanidine sulfate was isolated, and in the latter ammonia was detected.

(1) Presented in part before the Division of Medicinal Chemistry, 9th National Medicinal Chemistry Symposium of the American Chemical Society, Minneapolis, Minn., June 21–24, 1964.

(2) (a) J. E. Robertson, J. H. Biel, and F. DiPierro, *J. Med. Chem.*, **6**, 381 (1963); (b) E. G. Podrebarac, W. H. Nyberg, F. A. French, and C. C. Cheng, *ibid.*, **6**, 283 (1963); (c) J. H. Short, U. Biermacher, D. A. Dunnigan, and T. D. Leth, *ibid.*, **6**, 275 (1963); (d) C. Cipens and V. Grinsteins, *Zh. Obshch. Khim.*, **32**, 3811 (1962); (e) A. H. Greer and G. B. L. Smith, *J. Am. Chem. Soc.*, **72**, 874 (1950).

(3) Pfizer Ltd., Belgian Patent 629,613 (Oct. 2, 1963); *Chem. Abstr.*, **60**, 14437 (1964). During the preparation of guanoclor on a multi-kilogram scale, it was apparent from infrared spectroscopy that later fractions did contain traces of the isomer V, and a small quantity of the isomer was isolated by repeated fractional crystallization.

(4) *Brit. Med. J.*, **1**, 621 (1964); marketed in Great Britain as Vatensol®.

(5) J. Augstein and S. M. Green, *Nature*, **201**, 628 (1964); T. D. V. Lawrie, A. R. Lorimer, S. G. McAlpine, and H. Reinert, *Brit. Med. J.*, **1**, 402 (1964).