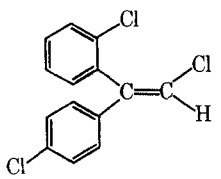
Assignment of Configuration of Dehydrochlorination Products of Mitotane

DAVID B. ROLL* and P. BELTRAME†

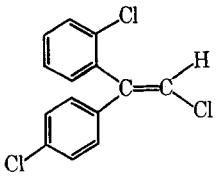
Abstract □ The drug of choice in the treatment of adrenal cortical carcinoma is mitotane (*o,p'*-DDD). The two geometric isomers obtained on dehydrochlorination of mitotane were separated for the first time and characterized. Analysis of the NMR spectra and the results of dipole moment measurements conclusively prove that the assignment of configuration of these compounds in the literature is incorrect. These compounds may be metabolites of mitotane.

Keyphrases □ Mitotane—separation, characterization, configuration assignment of dehydrochlorination products □ Configuration assignment—mitotane isomers, correction □ NMR spectroscopy—configuration, mitotane isomers

Approximately two people per million population are afflicted with cancer of the adrenal cortex (1). The drug of choice in the treatment of this condition is 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane [mitotane (*o,p'*-DDD)] a compound closely related in structure to the pesticide chlorophenothane (*p,p'*-DDT). The latter compound is readily dehydrochlorinated in the body to 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene (*p,p'*-DDE). Since the normal dose of mitotane is quite high, 10 g./day, it is possible that one of its metabolites, such as the dehydrochlorination products, could be responsible for the antitumor activity. Dehydrochlorination of mitotane leads to the formation of two geometric isomers: *cis*- and *trans*-1-chloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene (I and II), where the terms *cis* and *trans* refer to the relative orientations of the 1-chloro and 2-(*o*-chlorophenyl) groups. This report is concerned with the assignment of the configuration of these compounds using NMR and dipole moment measurements and conclusively shows that the previous structural assignments in the literature are incorrect (2, 3).



I



II

EXPERIMENTAL

Compounds I and II were prepared by dehydrochlorination of mitotane[1]. In a typical synthesis, 12.8 g. (0.04 mole) of mitotane was dissolved in 160 ml. of 1 *N* ethanolic NaOH, and the solution was refluxed for 1 hr. This was accompanied by the precipitation of NaCl. Water was added to the reaction mixture, and the resulting mixture was extracted with diethyl ether and dried with anhydrous calcium sulfate[2]. The solvent was removed under vacuum, the weight of the crude product being 95% of theoretical. The mixture was resolved into two components, with no indication of starting material by TLC on an alumina plate. Prior to chromatography, the plate was dipped in a solution of 1:3 DMF–diethyl ether, developed with 2,2,4-trimethylpentane, and sprayed with silver nitrate solution. The average R_f values for the isomers were 0.63 (I) and 0.77 (II), and they were present in the mixture in approximately equal concentrations.

The isomers were ultimately separated in gram quantities by column chromotography. In a typical separation, approximately 1 g. of the mixture of Isomers I and II dissolved in 50 ml. of petroleum ether was added to a 2 × 30-cm. column packed with 30 g. of neutral alumina. The column was eluted with petroleum ether, and 10 fractions of approximately 100 ml. each were collected. The separation of the components in the various fractions was followed by gas chromatography, employing an F and M model 700 unit equipped with a thermal conductivity detector using a 1.8 m. × 0.64-cm. (6 ft. × 0.25-in.) glass column packed with 3% diethylene glycol succinate on 80/100 mesh Gas Chrom Q at the following temperatures: injection port, 235°; oven, 190°; and detector, 230°. The flow rate of the helium carrier gas was 45 ml./min. The retention times under these conditions were approximately 9.5 (II) and 11 (I) min. Fractions rich in the individual isomers, I and II, were pooled and crystallized from hexane. Final crystallization from ethanol in the case of I and cyclohexane in the case of II resulted in melting points of 54–55° and 53.5–54°, respectively. Melting points are uncorrected and were determined on a Kofler micro hot stage.

Anal.—Calcd. for $C_{14}H_9Cl_3$; C, 59.30; H, 3.20. Found: (I) C, 59.26; H, 3.01. (II) C, 59.09, H, 3.34.

The NMR spectra were determined with a JNM-C-60H spectrometer. Spectra were taken in deuterochloroform in a concentration of about 100 mg./0.5 ml. with tetramethylsilane as the internal reference. The chemical shifts of the vinylic protons of I and II were 6.73 and 6.37 δ, respectively. The UV spectra were obtained on a Cary 14 spectrophotometer.

The dipole moments were obtained in dry benzene employing weight fractions of solute W_2 of approximately 0.005, 0.01, 0.015, and 0.02. The dielectric constants (e) were measured at 25° using a Dipolmeter DM 01 WTW, and refractive indexes (n) at the same temperature were determined with an Abbe refractometer. In each case, several readings were taken for the solution, the solvent, and the air in various sequences (4).

The orientation molar polarization of the solute P_2 was obtained by the Guggenheim method (5), plotting e and n^2 *versus* W_2 and evaluating the slopes of the graphs, α and ν, respectively. Plots were linear within experimental error; in each case, a straight regression line was obtained by the least-squares method. The value of P_2 was obtained from the equation

$$P_2 = 3\frac{M_2}{d_1}\frac{\alpha - \nu}{(e_1 + 2)^2} \qquad \text{(Eq. 1)}$$

where M_2 is the molecular weight of the solute, and d_1 and e_1 are the density and dielectric constant of the pure solvent, respectively. The dipole moment μ (in Debye) was calculated from P_2 (in milliliters/mole) by the equation

$$\mu = 0.01282\sqrt{P_2 T} \qquad \text{(Eq. 2)}$$

By using $d_1 = 0.87368$ and $e_1 = 2.2726$, the results in Table I were obtained.

RESULTS AND DISCUSSION

Configurational Assignment of I and II Using NMR—The compound 1-chloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene was first synthesized in 1962 (6). However, mention was not made of the possibility of two geometric isomers. More recently, Sharpless and Bradley (2) synthesized the mixture of I and II. Although they

[1] Aldrich Chemical Co. Inc., Milwaukee, Wis.
[2] Drierite.

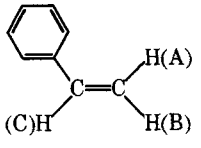
were unable to separate the isomers, they did obtain the NMR spectra which showed two sharp singlets in the vinylic region indicative of two nonequivalent vinylic protons. Their results showed that the aromatic portion of the spectrum was an unresolved multiplet and that the difference in chemical shift of the two vinylic signals was 0.34 p.p.m., which compares favorably with the difference of 0.36 p.p.m. in the current investigation. On the basis of the well-known fact that close proximity to other nuclei such as chlorine deshields protons (7, 8), they assigned the *trans*-configuration to the isomer in which the vinylic signal is the farther downfield. From an examination of a planar representation of the structure, this vinylic proton appears to be closer to the *o*-chloro group.

The same interpretation of the NMR results was given by Keith *et al.* (3). This interpretation of the NMR spectra, however, ignored possible differences in the chemical shift of the vinylic signals due to the magnetic anisotropy of the aromatic rings and differences in the preferred conformations of these rings in the two compounds. Indeed, when these factors are considered and in light of the dipole measurements, it is apparent that the assignment of structure made by these two groups is incorrect and that the isomer in which the vinylic signal is at the lowest field is actually the *cis*-isomer, I. This is the isomer in which, in the most favorable conformation, the ring *cis* to the vinylic hydrogen is in the same plane as the double bond, and the vinylic hydrogen is then in the region of maximum deshielding due to the magnetic anisotropy of the aromatic ring. An examination of molecular models indicates that there is more steric interference to a planar arrangement for the aromatic group *cis* to the vinylic proton in II than in I; thus the vinylic hydrogen in II is not deshielded as much and may actually be in the region of shielding. The difference in chemical shift for the signals of the vinylic protons in I and II of 0.36 p.p.m. is consistent with the downfield shift of 0.38 p.p.m. for the signal of the *cis*-β-proton in styrene as compared to ethylene (9).

The effect of the position of substitution on an aromatic ring and the resulting influence on the NMR of adjacent vinylic protons are shown by an examination of data obtained by Gurudata *et al.* on some aryl substituted styrenes (10). An examination of the chemical shift for the α-proton (Structure III, C) in the *o*-chloro-substituted derivative compared to the *p*-chloro compound shows that this proton is deshielded 0.45 p.p.m. in the former compound as compared to the latter. In compounds of this type, the preferred planar conformation is that in which the β-carbon is as far removed from the *ortho*-substituent as possible. The result is that the α-proton is in close proximity to the *ortho*-substituent, but not in such close proximity as to prevent a planar conformation and the α-proton is consequently deshielded. Thus, the *o*-chlorine is not adjacent to the *cis*-β-proton at all, but rather to the α-proton in these compounds.

It is instructive to consider the chemical shift of the A proton in the *ortho*- and *para*-chloro-substituted derivatives; there is very little difference in chemical shift for this proton in these two compounds with δ values of 5.59 and 5.67, respectively. This fact does not support the argument that proximity between the A proton and the chloro group is an important factor in the assignment of configuration in these compounds, and it shows that the aromatic ring has approximately the same degree of coplanarity with the vinylic group in the two compounds.

From an examination of molecular models of I and II, it is apparent that coplanarity of either ring with the vinyl group is exceedingly difficult in II. However, the *p*-chlorophenyl group in I has freedom of rotation and, therefore, can exist in the preferred planar orientation, resulting in deshielding of the *cis*-vinylic proton. Van der Linde *et al.* (11) obtained the NMR spectra of some 1,1-diarylalkenes. When one of the aryl groups carries an *ortho*-methyl substituent, the coplanarity of this ring is particularly disturbed and the absorption signal of the *cis*-hydrogen is shifted to higher field as compared to the compound in which both aryl groups are phenyl. The result is, however, that the other styrene system possessing only *ortho*-hydrogens can now more easily assume planarity, which then results in a downfield shift of 0.33 p.p.m. for the vinylic



III

Table I—Results of Dipole Moment Measurements for Isomers I and II

Parameter	I[a]	II[a]
α	1.541 ± 0.093	3.742 ± 0.089
ν	0.265 ± 0.019	0.259 ± 0.014
P_2 (milliliters/mole)	68.1 ± 5.1	185.8 ± 4.8
μ (Debye)	1.83 ± 0.07	3.02 ± 0.04

[a] Each figure is given with its probable error.

hydrogen *cis* to this phenyl group. The present situation is very similar; in I, coplanarity of the *p*-chlorophenyl group can occur, the result being the deshielding of the *cis*-vinylic proton. The UV data also support the idea of greater planarity in I (ϵ = 16,000, $\lambda_{max.}$ 258 nm.) than in II (ϵ = 14,900, $\lambda_{max.}$ 241 nm.).

The NMR signals for the aromatic portion of these molecules are also of interest but are difficult to interpret. The aromatic signals for I are a complex multiplet centered at about δ 7.32, whereas the analogous signal in II is essentially a singlet at 7.28. There may be greater deshielding of the *ortho*-hydrogens of the *p*-chlorophenyl ring by the vinylic group in I where a greater degree of coplanarity is possible, leading to a more complex signal. From inspection of molecular models, it appears that the most favorable conformation of the aromatic rings in II is that in which they are approximately perpendicular to the vinyl group. If this is true, the long-range effects of the aromatic rings on each other would be similar and the chemical shift of all aromatic protons signals would be nearly identical.

Configurational Assignment of I and II from Dipole Moment Measurements—Evaluation of the expected dipole moments of I and II by vector addition of bond moments (12) is easily done since only C—Cl bonds give a significant contribution, the moment of the H—$C_{vinylic}$ and H—$C_{aromatic}$ bonds being approximately zero. The value of the dipole moment of chlorobenzene (μ = 1.58) (13) was used for every C—Cl bond in I and II. Thus, for I, since the compensation of the C—Cl bonds is independent of rotation, the residual moment is that of the chlorobenzene molecule, *i.e.*, 1.58 D, which agrees well with the experimental value of 1.83 D. In the case of II, the vector addition of the Cl—$C_{vinylic}$ and *p*—Cl—C bonds always occurs in the vinylic plane, while the third vector (*o*—Cl—C bond) is out of this plane. In the limiting case of a 90° rotation, the vector addition in three-dimensional space gives a moment of 3.71 D. Even if the groups in II are planar, the calculated dipole moment would be 3.16 D. Thus, the calculated value for II varies between 3.16 and 3.71 D according to the amount of deviation from coplanarity, and these values are comparable to the experimental value of 3.02 D (Table I).

The experimental values obtained for I and II do not exactly match the calculated values because of certain approximations made in the calculations, such as the assumption of 120° angles and the additivity of bond moments, and neglect of any contribution from conjugation. Since the difference in the calculated dipole moments for the two isomers is large and the error introduced through the simplifying assumptions is small, there is no doubt concerning the assignment of configuration and the dipole moment measurements confirm the NMR results.

Compounds I and II are currently undergoing screening for antitumor activity at the Cancer Chemotherapy National Service Center.

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* To whom requests for reprints should be directed.

† Present address: Istituto di Chimica Fisica, Universita' di Milano, 20133 Milano, Italy.

TLC Analysis of Mannich Bases Derived from Indolizines

N. H. CHOULIS* and W. B. HARRELL†

Abstract □ Seven Mannich bases derived from indolizines were studied using TLC procedures. Alumina, cellulose, and silica gel plates and a number of solvent systems were employed.

Keyphrases □ Mannich bases, from indolizines—TLC studies □ TLC—characterization, Mannich bases derived from indolizines □ Indolizine Mannich base derivatives—TLC requirements, characterization

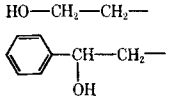
It was established in previous work that certain indolizines with dialkylaminomethyl side chains at the C-3 position exhibit pronounced CNS depression (1, 2). As part of a continuing exploration of indolizines with potential biological activity, a series of Mannich bases with *N*-substituted cyclohexylaminomethyl groupings were synthesized using 1,2-diphenylindolizine as the starting compound. The chemistry and biological activities of these compounds are reported elsewhere (3). In the present investigation, the characterization of these compounds is attempted using TLC procedures.

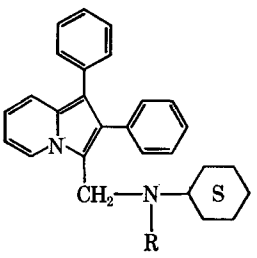
EXPERIMENTAL

Materials—3-Cyclohexylaminomethyl derivatives of 1,2-diphenylindolizine (Structure I) were used, where R was substituted as indicated in Table I. These compounds were prepared according to the methods described by Harrell *et al.* (3).

Developing Systems—Nineteen different solvent systems, indicated in Table II, were employed.

Table I—Compounds Used

Compound Number	R =	Melting Point
I		182–183°
II	$-C_2H_5$	123–124°
III	$-CH_3$	112–113°
IV	$(CH_3)_2CH-$	127–128°
V	$NC-CH_2-CH_2-$	152–153°
VI	$HO-CH_2-CH_2-$	138–139°
VII		137–138°



I

Detection—In most cases the spots were observed visually, giving a characteristic green color. The color was developed immediately after the plates were removed from the tank. A UV lamp[1] was also used. All compounds appeared to fluoresce under the longwave UV light.

Preparation of Plates—The plates (20 × 20 cm.) were coated with alumina[2], cellulose[3], or silica gel[4], 250 nm. thick, according to Stahl (4).

General Procedure—Solutions of the compounds were prepared by dissolving 10-mg. quantities into 3 ml. of acetone, chloroform, dioxane, or dimethylformamide (DMF). Samples of the solutions were applied 1.5 cm. from the bottom edges of the alumina, cellulose, or silica gel plates, and the ascending chromatograms were allowed to travel 10 cm. at room temperature. All experiments were repeated in a dark room at the same temperature (21°).

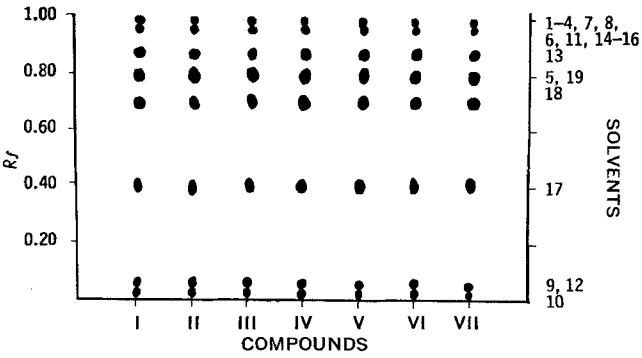


Figure 1—*Spot detection on silica gel plates. Left: R_f values. Right: solvent systems used (Table II). Compounds I–VII are those listed in Table I.*

[1] B. L. E. Spectroline, model C-3F.
[2] Aluminum Oxide G, Merck Co.
[3] MN-Cellulose Powder 300, Macherey, Nagel and Co.
[4] Silica gel G, Merck Co.