# Aromatic Hydroxylation and Alkyl Oxidation in Metabolism of Mitotane (*o,p'*-DDD) in Humans

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**Abstract** □ Ether extracts of urine from patients treated with mitotane (*o,p'*-DDD) were methylated with diazomethane. Five derivatives of metabolites were detected and identified by comparison of GLC retention times, mass spectra, and IR spectra with synthetic reference compounds. The derivatives are: methyl *o,p'*-dichlorodiphenylacetate, methyl 1-(2-chloro-4-methoxyphenyl)-1-(4-chlorophenyl)acetate, methyl 1-(2-chloro-3-methoxyphenyl)-1-(4-chlorophenyl)acetate, methyl 1-(2-chloro-3,4-dimethoxy)-1-(4-chlorophenyl)acetate, and *o,p'*-dichlorodiphenylacetylglycine methyl ester. The synthesis and mass, IR, and NMR spectra of reference compounds are given. Metabolite levels over a period of time in urine samples of two patients were quantitated by GLC.

**Keyphrases** □ Mitotane—aromatic hydroxylation and alkyl oxidation during metabolism, humans, detection and identification of metabolites □ *o,p'*-DDD (mitotane)—aromatic hydroxylation and alkyl oxidation during metabolism, humans, detection and identification of metabolites □ Metabolism—mitotane in humans, aromatic hydroxylation and alkyl oxidation, detection and identification of metabolites

Mitotane[1], 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane (*o,p'*-DDD) (I), has been shown to produce atrophy of the adrenal cortex, inhibition of ACTH stimulation of steroidogenesis, and alteration of extraadrenal cortisol metabolism (1). Its utility in the treatment of Cushing's syndrome secondary to either adrenal cortical carcinoma (2) or hyperfunction (3) has led to Food and Drug Administration (FDA) approval for human use, although knowledge is limited as to its mode of action, side effects, and biotransformation.

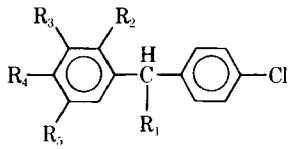
Mitotane shows a species difference in its effects on the adrenal cortex. It causes adrenal atrophy in dogs and humans, but it is inactive in rats, mice, rabbits, and monkeys (4). The compound can inhibit adrenal glucose 6-phosphate dehydrogenase activity in the dog and ACTH stimulation of steroidogenesis *in vivo* but not *in vitro* (1, 5). These findings might suggest that a metabolite of I rather than the compound itself is responsible for its activity.

In addition, 1,1-bis(*p*-chlorophenyl)-2,2-dichloroethane (*p,p'*-DDD)[2] (II) is relatively inactive, while 1-(*m*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane (*m,p'*-DDD)[3] (III) shows adrenolytic activity in the dog (6). A difference in lipid solubility (7), a free *para*-position, or a biotransformation to give a *para*-hydroxylated species might be responsible for the difference in activity. Biotransformation of an analogous compound, 2-chlorophenoxyacetic acid, in *Aspergillus niger* gives hydroxylation at primarily the 4- and 5-positions (8).

Previously, *o,p'*-dichlorodiphenylacetic acid (*o,p'*-DDA) (IV*a*) was identified in these laboratories as a metabolite of I in humans (9), but ring hydroxylation of I has not been reported. This study was undertaken to detect and identify other metabolites of I. Urine from patients with adrenal cortical carcinoma being treated with I was used as the source of these metabolites.

## RESULTS

Ether extracts of urine were divided into: (*a*) a neutral fraction, (*b*) a sodium hydroxide-soluble fraction, and (*c*) a sodium bicarbonate-soluble fraction (Scheme I). The fractions were methylated with diazomethane and examined by GLC–mass spectroscopy. A metabolite of I was believed to be present when unique GLC peaks were present in urine fractions from treated patients but not in the corresponding fractions from urine of control subjects. The chlorinated character of the compounds was initially indicated by their characteristic mass spectra in the molecular ion region of such compounds. No chlorinated compounds were found in the sodium hydroxide and neutral fractions. However, the presence of the



I: $R_1 = CHCl_2$; $R_2 = Cl$; $R_3$, $R_4$, $R_5 = H$
II: $R_1 = CHCl_2$; $R_4 = Cl$; $R_2$, $R_3$, $R_5 = H$
III: $R_1 = CHCl_2$; $R_3 = Cl$; $R_2$, $R_4$, $R_5 = H$

IV: $R_1 = CO_2CH_3$; $R_2 = Cl$; $R_3$, $R_4$, $R_5 = H$
IV*a*: $R_1 = CO_2H$; $R_2 = Cl$; $R_3$, $R_4$, $R_5 = H$

VII: $R_1 = CO_2CH_3$; $R_2 = Cl$; $R_3$, $R_4 = H$; $R_5 = OCH_3$
VII*a*: $R_1 = CO_2H$; $R_2 = Cl$; $R_3$, $R_4 = H$; $R_5 = OH$

VIII: $R_1 = CO_2CH_3$; $R_2 = Cl$; $R_3$, $R_5 = H$; $R_4 = OCH_3$
VIII*a*: $R_1 = CO_2H$; $R_2 = Cl$; $R_3$, $R_5 = H$; $R_4 = OH$

X: $R_1 = CO_2CH_3$; $R_2 = OCH_3$; $R_3$, $R_5 = H$; $R_4 = Cl$
X*a*: $R_1 = CO_2H$; $R_2 = OH$; $R_3$, $R_5 = H$; $R_4 = Cl$

XI: $R_1 = CO_2CH_3$; $R_2 = Cl$; $R_3 = OCH_3$; $R_4$, $R_5 = H$
XI*a*: $R_1 = CO_2H$; $R_2 = Cl$; $R_3 = OH$; $R_4$, $R_5 = H$

XII: $R_1 = CO_2CH_3$; $R_2 = Cl$; $R_3 = H$; $R_4$, $R_5 = OCH_3$
XII*a*: $R_1 = CO_2H$; $R_2 = Cl$; $R_3 = H$; $R_4 = OCH_3$; $R_5 = OH$
XII*b*: $R_1 = CO_2H$; $R_2 = Cl$; $R_3 = H$; $R_4 = OH$; $R_5 = OCH_3$

XIII: $R_1 = CO_2CH_3$; $R_2 = Cl$; $R_3$, $R_4 = OCH_3$; $R_5 = H$
XIII*a*: $R_1 = CO_2H$; $R_2 = Cl$; $R_3 = OCH_3$; $R_4 = OH$; $R_5 = H$
XIII*b*: $R_1 = CO_2H$; $R_2 = Cl$; $R_3 = OH$; $R_4 = OCH_3$; $R_5 = H$

XIV: $R_1 = CONHCH_2CO_2CH_3$; $R_2 = Cl$; $R_3$, $R_4$, $R_5 = H$
XIV*a*: $R_1 = CONHCH_2CO_2H$; $R_2 = Cl$; $R_3$, $R_4$, $R_5 = H$

[1] Preferred USAN chemical name is 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane.
[2] Preferred Merck chemical name is 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane.
[3] Preferred Merck chemical name is 1,1-dichloro-2-(*m*-chlorophenyl)-2-(*p*-chlorophenyl)ethane.

**Table I**—GLC Retention Times (Minutes) of Methylated Metabolites[a]

| Column | o,p'-Dichlorodiphenylacetic Acid Methyl Ester (IV) | Bis(p-chlorophenyl)acetic Acid Methyl Ester[b] | Derivatives of o,p'-Dichlorodiphenylacetic Acid (IVa) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 5-Methoxy (VII) | 4-Methoxy (VIII) | 3-Methoxy (XI) | 4,5-Methoxy (XII) | 3,4-Methoxy (XIII) | Glycine Conjugate (XIV) |
| 3% OV-1[c] | 6.5 | 7.4 | 11.3 | 11.3 | 11.3 | 14.5 | 14.5 | 18.7 |
| 3% OV-17[d] | 24.3 | —[e] | 32.6 | 34.1 | 35.5 | 39.7 | 40.7 | —[f] |
| 3% OV-25[d] | 25.6 | —[e] | 33.9 | 36.2 | 38.5 | 42.6 | 44.1 | —[f] |

[a] Retention times are for reference standards that cochromatograph with urine extract peaks. [b] Internal standard. [c] Column temperature at 190° for 4 min followed by a 4°/min program. See text, Footnote 6, for other conditions. [d] Column temperature at 170° with a 2°/min program. See text, Footnote 6, for other conditions. [e] Not determined in this system. [f] Glycine conjugate is not detected under these conditions.

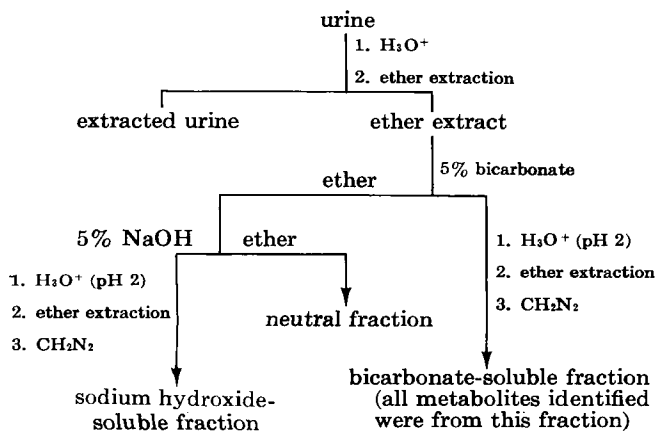
methyl ester of o,p'-dichlorodiphenylacetic acid (IV) was detected as arising from the sodium bicarbonate fraction. The methyl derivatives of hydroxylated, dihydroxylated, and glycine-conjugated derivatives of IVa were also detected. The structures of these derivatives were determined by comparison of GLC retention time, mass spectra, and IR spectra with synthetic reference compounds.

Condensation of the appropriate chloromethoxymandelic acids (V and VI) with chlorobenzene was attempted to synthesize the 5-methoxy-substituted (VII) and the 4-methoxy-substituted (VIII) methyl esters of o,p'-dichlorodiphenylacetic acid. This procedure was analogous to the reported (10) synthesis of the methyl ester of o,p'-dichlorodiphenylacetic acid. Preparation of the mandelic acids was similar to that reported for mandelic acid (11).

The preparation of the 5-methoxy compound (VII) was successful, but the condensation of 2-chloro-4-methoxymandelic acid with chlorobenzene failed to yield any material that could be identified as VIII, the 4-methoxy-substituted compound. However, VIII was obtained by methylation of a condensation product of 4-chloromandelic acid with 3-chlorophenol. As anticipated, substitution also occurred *para* to the chloro group of 3-chlorophenol. This product was present as the lactone of 2-hydroxy-substituted bis(p-chlorophenyl)acetic acid (Xa) in the reaction fraction, which is insoluble in sodium bicarbonate solution. Compound Xa, characterized as the methylated Compound X, is of interest since it is a potential metabolite of the insecticide DDT[4] [1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane, p,p'-DDT]. Hydroxylation at the 2-position of 4-chlorophenoxyacetic acid has been reported (8).

Figure 1 shows a GLC separation on an OV-25 column of a typical methylated urine extract. The relatively small peak B (less than 10% of C) observed in several bicarbonate-soluble urine fractions cochromatographed with the synthetic 5-methoxy compound (VII). However, insufficient amounts were present to confirm these results by mass spectroscopy.

Peak C corresponded in GLC retention time as well as in its mass spectrum to the synthetic 4-methoxy compound (VIII). A urine extract in which the ratio of D to C was less than 5% was used to isolate C by preparative GLC. The IR spectra from this material corresponded to that of synthetic VIII but not VII or XI.




*Scheme I—Partition of urine ether extract*

Acetylation of the urine extract followed by methylation (12) gave a GLC peak whose mass spectrum indicated the methyl esterified, *O*-acetoxy derivative of the 4-hydroxy compound (VIIIa). However, no Compound VIII was detected, thus indicating that Metabolite C is actually present in urine as VIIIa and not its methoxy derivative.

The mass spectrum of peak D was similar to that of peak C except for the addition of a fragment at mass 289, which indicated loss of one chlorine from the parent compound. The GLC retention time and mass spectrum of the synthetic 3-methoxy compound[5] (XI) (13) were identical to those of peak D.

The methylated derivative of the dihydroxy metabolite present in the urine extract was originally thought to be XII, the 4,5-dimethoxy isomer, based upon GLC on an OV-1 column. However, cochromatography of synthetic XII with the urine extract on OV-17 and OV-25 columns indicated a retention time corresponding to peak E of Fig. 1 and not F, the dimethoxy metabolite peak. Compound XIII, the 3,4-disubstituted isomer, synthesized by Feil *et al.* (13), cochromatographed with peak F. Peak E may be the 4,5-isomer present in amounts of less than 15% of F. However, because of the low amounts present and its close proximity to F, it was not possible to confirm E to be the dimethoxy Compound XII by mass spectroscopy.

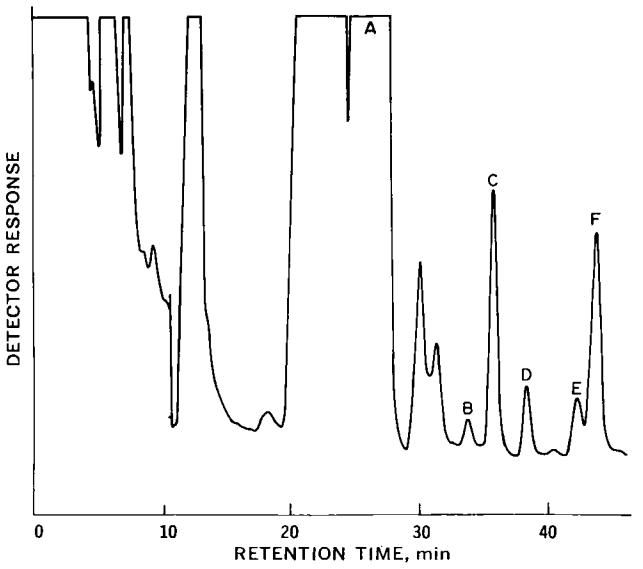



**Figure 1**—*GLC trace of a typical urine ether extract with retention times in minutes, OV-25 column at 170° with 2°/min program. The indicated peaks cochromatographed with the following compounds: A, methyl* o,p'-*dichlorodiphenylacetate (IV); B, methyl 1-(2-chloro-5-methoxyphenyl)-1-(4-chlorophenyl)acetate (VII); C, methyl 1-(2-chloro-4-methoxyphenyl)-1-(4-chlorophenyl)acetate (VIII); D, methyl 1-(2-chloro-3-methoxyphenyl)-1-(4-chlorophenyl)acetate (XI); E, methyl 1-(2-chloro-4,5-dimethoxyphenyl)-1-(4-chlorophenyl)-acetate (XII); and F, methyl 1-(2-chloro-3,4-dimethoxyphenyl)-1-(4-chlorophenyl)acetate (XIII).*

[4] Preferred Merck chemical name is 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.

[5] Supplied by V. Feil, U. S. Department of Agriculture.

**Table II**—Quantitation by GLC of Metabolites of Mitotane in Urine from Patient 1 (M.B.)[a]

| Days of Administration | *o,p'*-Dichlorodiphenylacetic Acid (IV*a*) | *o,p'*-Dichlorodiphenylacetic Acid Derivatives | | | |
|---|---|---|---|---|---|
| | | Monohydroxy (VIII*a* + XI*a*) | Hydroxy Methoxy (XIII*a* + XIII*b*) | Glycine Conjugate (XIV*a*) | Total |
| 134 | 0.045 (18.2) | 0.015 (5.9) | 0.010 (3.5) | 0.011 (3.8) | 0.081 (31.4) |
| 134[b] | 0.073 (29.9) | 0.051 (19.5) | 0.020 (7.1) | 0 (0) | 0.144 (56.5) |
| 253 | 0.034 (10.0) | 0.024 (6.6) | 0.015 (3.8) | 0.014 (3.5) | 0.087 (23.9) |
| 290 | 0.021 (7.7) | 0.008 (2.9) | 0.011 (3.7) | 0.012 (3.8) | 0.052 (18.1) |
| 399 | 0.060 (11.8) | 0.017 (3.2) | 0.019 (3.3) | 0.013 (2.9) | 0.109 (21.2) |
| 401 | 0.046 (11.9) | 0.017 (4.2) | 0.038 (8.6) | 0.028 (6.3) | 0.129 (31.0) |

[a] Values are milligrams of metabolite per milligram of creatinine excreted. Values in parentheses represent percent excretion of the daily dose of 2 g. [b] Values found after acid hydrolysis of urine.

It is difficult to differentiate between the mass spectra of the dimethoxy isomers XII and XIII. Likewise, the IR spectra of synthetic XII, synthetic XIII, and the metabolite isolated by preparative GLC are similar. A discernable difference is that both the metabolite and XIII have IR bands at 1270 and 1290 $cm^{-1}$ while XII has bands at 1310, 1270, and 1250 $cm^{-1}$.

Acetylation of urine extract followed by methylation gave a GLC peak whose mass spectrum indicated the methyl esterified, *O*-acetoxy derivative of XIII*a* and/or XIII*b*. The literature (13–15) suggests that XIII would most likely be originally present in urine as one or both of the monomethylated catechols XIII*a* and XIII*b*.

The IR and mass spectrum and the GLC retention time of synthetic methylated glycine conjugate XIV are identical to those of the compound in urine extracts, confirming the presence of the glycine conjugate of *o,p'*-dichlorodiphenylacetic acid as a urinary metabolite of mitotane.

Table I gives GLC retention times on three different columns for peaks in the urine extract that cochromatographed with the appropriate synthetic standards and, except in the cases of VII and XII, were confirmed with the appropriate mass spectra. In these programmed temperature determinations, an increase in height of a particular peak when a synthetic compound was added to and coinjected with the urine extract was a more reliable indicator of equal retention times than separate determinations. The first set of conditions was used for metabolite quantitation and includes the retention time for the methyl ester of the internal standard bis(*p*-chlorophenyl)acetic acid. Recovery of the internal standard through the extraction, methylation, and chromatographic processes was 22.4–55.6%.

Tables II and III list amounts of the four metabolites quantitated by GLC from human urine extracts. Both patients were female and suffering from adrenal carcinoma. Patient 1 (M.B.) was given 6 g of mitotane/day for 85 days, at which time the dose was lowered to 2 g/day. Patient 2 (N.P.) started with a dose of 6 g/day and after 145 days the regimen was changed to 2 g/day. The monohydroxy values given represent a sum of the 4-hydroxy (VIII) and 3-hydroxy (XI) compounds present. When using GLC conditions that separate VIII and XI, the percent of monohydroxy present as XI in several urine extracts ranged from less than 5 to 20%.

## DISCUSSION

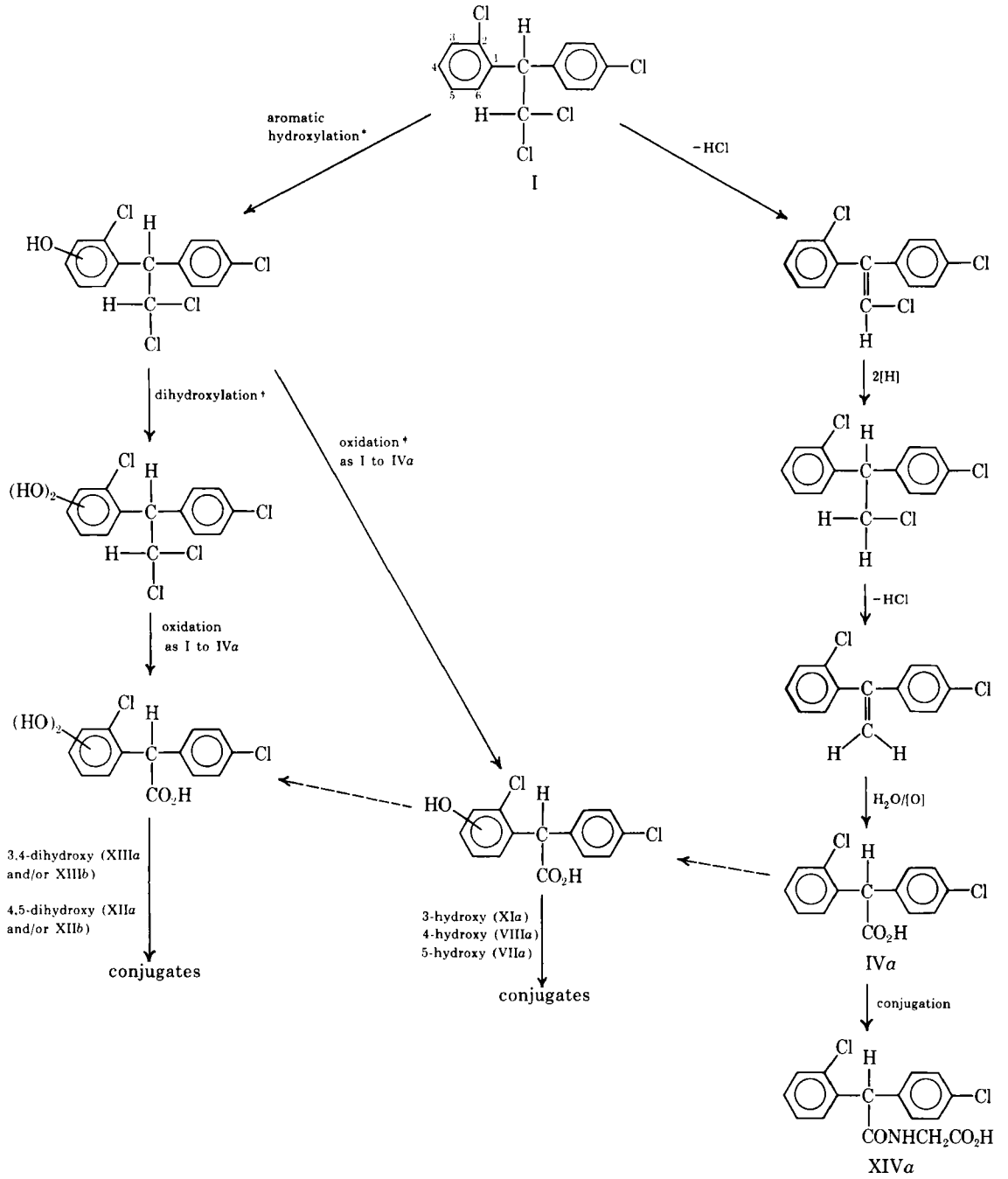
Recently, Feil *et al.* (13) identified a number of metabolites of the related compound 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane (*o,p'*-DDT) in rat feces which could also be metabolites of mitotane. They reported the hydroxylated carboxylic acid derivatives VII*a*, VIII*a*, XI*a*, and XIII*a*. Also reported were hydroxylated metabolites with an intact dichloro side chain and serine and glycine conjugates of the carboxylic acid IV*a*.

Based upon their work and that previously reported for analogous *o,p'*- and *p,p'*-dichlorodiphenyl-chlorinated ethane insecticides, it is reasonable to postulate the metabolic pathways for mi-

**Table III**—Quantitation by GLC of Metabolites of Mitotane in Urine from Patient 2 (N.P.)[a]

| Days of Administration | *o,p'*-Dichlorodiphenylacetic Acid (IV*a*) | *o,p'*-Dichlorodiphenylacetic Acid Derivatives | | | |
|---|---|---|---|---|---|
| | | Monohydroxy (VIII*a* + XI*a*) | Hydroxy Methoxy (XIII*a* + XIII*b*) | Glycine Conjugate (XIV*a*) | Total |
| 15 | 0.006 (1.0) | 0.002 (0.4) | 0 (0) | 0 (0) | 0.008 (1.4) |
| 21 | 0.077 (3.1) | 0.036 (1.4) | 0.037 (1.3) | 0.027 (0.9) | 0.177 (6.7) |
| 34 | 0.013 (1.2) | 0.006 (0.5) | 0.005 (0.4) | — | 0.024 (2.1) |
| 100 | 0.012 (1.2) | 0.005 (0.5) | 0.006 (0.5) | 0.005 (0.4) | 0.028 (2.6) |
| 100[b] | 0.051 (5.0) | 0.020 (1.9) | 0.039 (3.4) | 0 (0) | 0.110 (10.3) |
| 113 | 0.034 (2.2) | 0.015 (0.9) | 0.014 (0.8) | 0.010 (0.6) | 0.073 (4.5) |
| 154 | 0.022 (3.8) | 0.010 (1.6) | 0.009 (1.3) | 0.009 (1.3) | 0.050 (8.0) |
| 168 | 0.017 (5.8) | 0.003 (0.9) | 0.003 (1.0) | 0.011 (3.2) | 0.034 (10.9) |
| 168[b] | 0.075 (24.6) | 0.023 (7.0) | 0.052 (14.8) | 0 (0) | 0.150 (46.4) |

[a] Values are milligrams of metabolite per milligram creatinine excreted. Values in parentheses represent percent excretion of the dose given on that day. Daily dose was 6 g until Day 145 when it was reduced to 2 g because of severe GI side effects to the patient. [b] Values found after acid hydrolysis of urine.




*Scheme II—Possible metabolic pathways for mitotane. Compounds IV*a, *VII*a, *VIII*a, *XI*a, *XII*a, *XII*b, *XIII*a, *XIII*b, *and XIV*a *are indicated in the present study.* * *Hydroxylation has been reported at the 3- or 5-position and* † *dihydroxylation has been reported at 3- and 4-positions for the metabolism of 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2,2-trichloroethane in the rat (13).* ‡ *Dehalogenation and oxidation as reported for the chlorinated phenylethane insecticides (16).*

totane to be as outlined in Scheme II. This scheme is supported in the present study with indications for the presence of the carboxylic acid derivatives IV*a*, VII*a*, VIII*a*, XI*a*, XII*a*, XII*b*, XIII*a*, and XIII*b* as urinary metabolites of mitotane in humans. Unchanged mitotane was not detected in the urine. No metabolites with alkyl side chains intact nor intermediates toward oxidation to the carboxylic acid metabolites were isolated. Also there is no information as to relative extent of hydroxylation before or after side-chain oxidation.

Hydroxylation is primarily at the 4-position (VIII*a*) but is also at the 3-position (XI*a*). Dihydroxylation results in the 3,4-disubstituted isomers XIII*a* and/or XIII*b*. The presence of small amounts of 5-hydroxy (VII*a*) and 4,5-dihydroxy (XII*a* and/or XII*b*) metabolites is indicated by GLC studies only.

Acid hydrolysis of urine (Tables II and III) prior to extraction led to a higher percentage value of excretion for *o,p'*-dichlorodiphenylacetic acid (IV*a*) and its hydroxylated derivatives. Conjugates, other than the glycine derivative of IV*a* (XIV*a*) actually isolated, are indicated by the hydrolysis data. That is, the glycine conjugate does not account for the total increase of IV*a* nor the increase in the hydroxylated metabolites VIII*a* and XIII*a* or XIII*b* after hydrolysis.

Such conjugation is consistent with literature reports for analogous compounds. Thus, in addition to the serine and glycine conjugates of the carboxylic acid IV*a* reported by Feil *et al.* (13), serine and aspartic acid conjugates of bis(*p*-chlorophenyl)acetic acid have been reported (17) in the rat. Also the mouse and hamster are reported to conjugate this compound with alanine and glycine (18).

The quantitation data obtained did not exhibit any outstanding trends such as the appearance or disappearance of a given metabo-

lite. The variation in metabolite yield could be related to the method of analysis used and other factors such as fluctuating health, physical activity, and additional therapy received by the patient. Metabolite yields were expressed as a function of the creatinine excreted to correct for possible differences in renal clearance. These data show variations similar to that calculated as percent yield of daily dose administered.

In Patient 1, total metabolites detected before hydrolysis ranged from 18 to 31% of the dose administered on the day of urine collection. In Patient 2, they ranged from 1 to 11%. Hydrolysis data indicated a 56.5% total excretion for Patient 1; for Patient 2, excretions of 10.3 and 46.4% were indicated. These values are higher than Moy's (19) report that 10% of an oral daily dose of mitotane was excreted in the urine. He attributed 60% of the dose to unabsorbed material, with the remainder explained by tissue storage and biliary excretion.

## EXPERIMENTAL[6]

**2-Chloro-5-methoxymandelic Acid (V)**—A saturated sodium bisulfite solution (20 ml) was added over 10 min to a stirred mixture of 3.0 g (0.059 mole) of sodium cyanide, 10 ml of water, and 10.0 g (0.059 mole) of 2-chloro-5-methoxybenzaldehyde (20). During this addition, 18.0 g of ice was added. The mandelonitrile layer was separated and placed in concentrated hydrochloric acid at room temperature for 18 hr, with subsequent boiling for 5 hr. The hydrolysis solution was extracted with ether, the ether was removed *in vacuo*, and the residue was recrystallized three times from benzene to give white crystals, mp 102–103.5°, 7.32 g (57.6%); IR (KBr): 3400 (OH), 3000, 1700 (C=O), 1575, 1460, 1425, 1250, 1175, 1140, 1100, 1030, 925, 855, 815, 725, 645, and 605 $cm^{-1}$; NMR (acetone-$d_6$): $\delta$ 7.40 (1H, d, $J_{4,3}$ = 9.0 Hz, ArH), 7.22 (1H, d, $J_{4,6}$ = 3.0 Hz, ArH), 6.95 (1H, d of d, $J_{3,4}$ = 9.0 Hz, $J_{6,4}$ = 3.0 Hz, ArH), 5.63 (1H, s, CH), and 3.82 (3H, s, $OCH_3$).

*Anal.*—Calc. for $C_9H_9ClO_4$: C, 49.90; H, 4.19. Found: C, 49.78; H, 4.02.

**2-Chloro-4-methoxymandelic Acid (VI)**—The same procedure as for V was used. Amounts of reactants were 0.75 g (0.015 mole) of sodium cyanide, 2.49 ml of water, 2.50 g (0.015 mole) of 2-chloro-4-methoxybenzaldehyde (21), 4.25 ml of saturated sodium bisulfite solution, and 4.5 g of ice. The crude VI obtained was recrystallized three times in benzene to give white crystals, mp 108.5–110.0°, 1.26 g (39.3%); IR (KBr): 3460 (OH), 3070, 1730 (C=O), 1590, 1470, 1300, 1245, 1190, 1070, 1045, 930, 890, 820, 730, 710, 610, and 490 $cm^{-1}$; NMR (acetone-$d_6$): $\delta$ 7.53 (1H, d, $J_{5,6}$ = 8.0 Hz, ArH), 7.03 (1H, d, $J_{5,3}$ = 2.5 Hz, ArH), 6.98 (1H, d of d, $J_{6,5}$ = 8.0 Hz, $J_{3,5}$ = 2.5 Hz, ArH), 5.58 (1H, s, CH), and 3.85 (1H, s, $OCH_3$).

*Anal.*—Calc. for $C_9H_9ClO_4$: C, 49.90; H, 4.19. Found: C, 49.70; H, 4.29.

**Methyl 1-(2-Chloro-5-methoxyphenyl)-1-(4-chlorophenyl)acetate (VII)**—A flask, with magnetic stirring bar, containing a mixture of 2.32 g (0.0107 mole) of V and 1.44 g (0.0129 mole) of chlorobenzene was placed in an ice bath and 5 ml of concentrated sulfuric acid was added over 15 min. After 1 hr the ice bath was removed and the reaction was continued for 2.5 hr at room temperature. Ice was added to the flask, the mixture was extracted with ether, and the ether layer was extracted with 3 × 30 ml of 5% $NaHCO_3$. The sodium bicarbonate was acidified and extracted with ether, which was dried over sodium sulfate and treated with ethereal diazomethane. The ether was removed *in vacuo* and the residue was distilled, bp 165–170° (0.500 mm), 1.33 g (39.1%); IR (NaCl plates): 3010, 2950, 2840, 1725 (C=O), 1575, 1550, 1450, 1300, 1200, 1165, 1095, 1065, 1030, 1025, 815, 755, 635, and 505 $cm^{-1}$; NMR ($CDCl_3$): $\delta$ 7.31 (4H, s, ArH), 7.31 (1H, d, $J_{4,3}$ = 8.5 Hz, ArH), 6.90 (1H, d, $J_{4,6}$ = 3.0 Hz, ArH), 6.76 (1H, d of d, $J_{3,4}$ = 8.5 Hz, $J_{6,4}$ = 3.0 Hz, ArH), 5.47 (1H, s, CH), 3.72 (1H, s, $OCH_3$), and 3.70 (1H, s, $OCH_3$); mass spectroscopy (70 ev): *m/e* (relative intensity) 326 (26) M + 2, 325 (7) M + 1, 324 (38) $M^+$, 291 (7), 289 (21) $M^+$ − Cl, 267 (65), 266 (8), 265 (100) M − $C_2H_3O_2$, 232 (4), 231 (5), 230 (12), 229 (12), 217 (5), 215 (15), 195 (33), 186 (12), 163 (4), and 152 (34).

*Anal.*—Calc. for $C_{16}H_{14}Cl_2O_3$: C, 59.10; H, 4.34. Found: C, 59.15; H, 4.34.

**Methyl 1-(2-Chloro-4-methoxyphenyl)-1-(4-chlorophenyl)-acetate (VIII)**—In a mortar, 4.95 g (0.039 mole) of 3-chlorophenol and 6.00 g (0.032 mole) of 4-chloromandelic acid were mixed and then transferred to a flask placed in ice. Over 15 min, 15 ml of concentrated sulfuric acid was added while the mixture was stirred with a glass rod. The reaction was continued in ice for 1 hr and at room temperature for 3 hr with magnetic stirring. Workup was as in the preparation of VII. Treatment of the sodium bicarbonate-soluble fraction with diazomethane overnight gave a residue of 833 mg after solvent evaporation. With the aid of a Craig microdistillation apparatus, a fraction, bp 170–180° (0.500 mm), was obtained and recrystallized three times with methanol to give VIII, mp 82.5–84.0°; IR (KBr): 3100, 2950, 1710 (C=O), 1575, 1550, 1460, 1300, 1235, 1210, 1165, 1095, 1045, 1020, 1000, 860, 845, 765, 685, 565, and 495 $cm^{-1}$; NMR ($CDCl_3$): $\delta$ 7.32 (4H, s, ArH), 7.24 (1H, d, $J_{5,6}$ = 7.0 Hz, ArH), 7.01 (1H, d, $J_{5,3}$ = 2.5 Hz, ArH), 6.82 (1H, d of d, $J_{3,5}$ = 2.5 Hz, $J_{6,5}$ = 7.0 Hz, ArH), 5.43 (1H, s, CH), 3.80 (3H, s, $OCH_3$), and 3.77 (3H, s, $OCH_3$); mass spectroscopy (70 ev): *m/e* (relative intensity) 326 (13) M + 2, 325 (6) M + 1, 324 (19) $M^+$, 267 (67), 266 (17), 265 (100) M − $C_2H_3O_2$, 231 (9), 230 (8), 229 (13), 199 (5), 187 (15), 186 (11), 165 (5), 163 (4), and 152 (4).

*Anal.*—Calc. for $C_{16}H_{14}Cl_2O_3$: C, 59.10; H, 4.34. Found: C, 58.91; H, 4.40.

The crude methylated sodium bicarbonate-soluble fraction contained 65.6 mg of VIII (0.63% yield) as quantitated by GLC (OV-17 column at 250°). The fraction also contained 148.6 mg of methyl 4-chlorobenzoate, identified by its mass spectrum and GLC retention time comparisons and quantitated on OV-17 at 140° with 2°/min program.

The crude unmethylated sodium bicarbonate-soluble fraction was chromatographed on commercial 2-mm × 20-cm × 20-cm silica gel F-254 preparative TLC plates[7] with an acetic acid–chloroform (4:96 v/v) solvent system and development twice to 15 cm. Three bands and material at the origin were detected *via* fluorescent quenching at 254 nm. After separation on four plates with 100 mg of material on each, the bands were extracted with acetone and the solvent was removed. Residue from band one ($R_f$ 0.64) sublimed to give 48.3 mg of 4-chlorobenzoic acid, identified by comparing its NMR and IR spectra with commercial material. The residue from band three ($R_f$ 0.15) was isolated as an oil. The NMR ($CDCl_3$) indicated VIII*a*: $\delta$ 7.40 (4H, s, ArH), 7.25 (1H, d, $J$ = 8.5 Hz, ArH), 7.03 (1H, d, $J$ = 8.5 Hz, ArH), 6.87 (1H, d of d, $J$ = 2.5 Hz, $J$ = 8.5 Hz, ArH), and 5.47 (1H, s, CH). Methylation of the residue with diazomethane and crystallization with methanol gave 24.7 mg of VIII.

**1-(2-Hydroxy-4-chlorophenyl)-1-(4-chlorophenyl)acetic Acid Lactone (IX)**—The ether fraction remaining after the sodium bicarbonate extraction in the preparation of VIII was dried over sodium sulfate. The ether was removed *in vacuo* and the residue was recrystallized three times with methanol, mp 119–121°, 1.91 g (21.4%); IR (KBr): 3100, 1795 (C=O), 1600, 1460, 1230, 1195, 1180, 1135, 1090, 1055, 1015, 920, 855, 810, 800, and 500 $cm^{-1}$; NMR ($CDCl_3$): $\delta$ 7.28 (7H, m, with major peaks at 7.34, 7.23, and 7.22, ArH) and 4.87 (1H, s, CH); mass spectroscopy (70 ev): *m/e* (relative intensity) 280 (12) M + 2, 279 (4) M + 1, 278 (18) $M^+$, 251 (12), 250 (4), 249 (17) M − CHO, 217 (33), 216 (15), 215 (100) M − CO − Cl, 201 (2), 199 (16), 186 (5), 163 (4), 152 (28), and 125 (8).

*Anal.*—Calc. for $C_{14}H_8Cl_2O_2$: C, 60.24; H, 2.89. Found: C, 60.12; H, 2.91.

**Methyl 1-(2-Methoxy-4-chlorophenyl)-1-(4-chlorophenyl)acetate (X)**—Compound IX (1.91 g) was refluxed in 5% NaOH for 5 min. The sodium hydroxide was acidified with 10% HCl and

[6] A Loenco 160 GLC instrument equipped with flame-ionization detector and a 3% OV-17 on Gas Chrom Q (100–120) stainless steel column [1.8 m × 0.3 cm (6 ft × 0.125 in.)], with 30 ml/min helium carrier gas flow, was used where retention time determinations and quantitation involved use of this column. A 3% OV-25 on Chromasorb G (100–120) stainless steel column [1.8 m × 0.3 cm (6 ft × 0.125 in.)], with 30 ml/min helium carrier gas flow, was also used for retention time determinations. Preparative work was on a Varian Aerograph 90-P equipped with thermocouple detector and a 3% SE-30 on Gas Chrom Q (100–120) stainless steel column [1.5 m × 0.6 cm (5 ft × 0.25 in.)], with a helium carrier flow of 60 ml/min. Quantitation of metabolite levels was on a Hewlett-Packard 5750 equipped with a flame detector and a 3% OV-1 on Gas Chrom Q (100–120) stainless steel column [1.8 m × 0.3 cm (6 ft × 0.125 in.)], with a helium carrier flow rate of 65 ml/min. A DuPont 21-490 single-focusing instrument with the Loenco 160 GLC instrument was used for GLC–mass spectroscopy determinations.

[7] Brinkmann.

extracted with ether. The ether layer was dried over sodium sulfate, the solvent was removed, and the residue was isolated as an oil. NMR ($CDCl_3$) indicated Xa: δ 7.32 (4H, s, ArH), 6.87 (3H, m, ArH), and 5.18 (1H, s, CH). Diazomethane treatment of the residue and recrystallization four times with methanol gave crystals, mp 75–77°, 1.68 g (74.7% based on IX); IR (KBr): 3100, 2950, 1700 (C=O), 1560, 1475, 1375, 1305, 1250, 1215, 1165, 1115, 1090, 1030, 1020, 1000, 890, 845, 770, and 500 $cm^{-1}$; NMR ($CDCl_3$): δ 7.33 (4H, s, ArH), 7.02 (1H, s, ArH), 6.98 (1H, d, $J$ = 2.0 Hz, ArH), 6.93 (1H, d, $J$ = 2.0 Hz, ArH), 5.27 (1H, s, CH), 3.83 (3H, s, $OCH_3$), and 3.75 (3H, s, $OCH_3$); mass spectroscopy (70 ev): *m/e* (relative intensity) 326 (19) M + 2, 325 (6) M + 1, 324 (29) $M^+$, 295 (8), 293 (12) M − $CH_3O$, 267 (68), 266 (19), 265 (100) M − $C_2H_3O_2$, 251 (6), 249 (7), 231 (3), 230 (3), 229 (5), 217 (6), 215 (17), 199 (8), 197 (4), 195 (4), 186 (7), 165 (21), 152 (19), and 125 (16).

*Anal.*—Calc. for $C_{16}H_{14}Cl_2O_3$: C, 59.10; H, 4.34. Found: C, 59.10; H, 4.27.

**Methyl 1-(2-Chloro-4,5-dimethoxyphenyl)-1-(4-chlorophenyl)acetate (XII)**—Following the procedure used for VII, 2.00 g (0.0107 mole) of 4-chloromandelic acid and 2.22 g (0.0129 mole) of 4-chloroveratrole, prepared by dimethyl sulfate methylation of 4-chlorocatechol (22), were used. The sodium bicarbonate-soluble residue obtained was sublimed under vacuum and recrystallized twice with isopropanol to give white crystals, mp 81–83°, 1.32 g (38.3%); IR (KBr): 2950, 1725 (C=O), 1475, 1375, 1310, 1270, 1250, 1210, 1160, 1090, 1030, 1010, 965, 925, 860, 840, 780, and 755 $cm^{-1}$; NMR ($CDCl_3$): δ 7.30 (4H, s, ArH), 6.94 (2H, s, ArH), 5.47 (1H, s, CH), 3.86 (3H, s, $OCH_3$), 3.80 (3H, s, $OCH_3$), and 3.78 (3H, s, $OCH_3$); mass spectroscopy (70 ev): *m/e* (relative intensity) 356 (17) M + 2, 355 (4) M +1, 354 (26) $M^+$, 324 (3), 322 (4), 297 (65), 295 (100) M − $C_2H_3O_2$, 261 (4), 259 (9) M − $C_2H_3O_2$ − HCl, 247 (4), 245 (13) M − $ClC_2H_3O_2$ − $CH_3$, 255 (13), 202 (7), 199 (9), 182 (9), 181 (9), 173 (9), 152 (11), 139 (17), and 125 (13).

*Anal.*—Calc. for $C_{17}H_{16}Cl_2O_4$: C, 57.48; H, 4.54. Found: C, 57.34; H, 4.59.

***o,p'*-Dichlorodiphenylacetyl Glycine Methyl Ester (XIV)**—Starting material IVa was synthesized according to literature methods (10). A solution of 0.35 g (0.00125 mole) of IVa, 0.16 g (0.00125 mole) of glycine methyl ester hydrochloride, and 0.054 g (0.00125 mole) of triethylamine in 25 ml of benzene–methanol (4:1) was added to 0.32 g (0.0013 mole) of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (23). The solution was stirred at room temperature for 5 hr, washed with 10 ml of water, and then washed with 2 × 10 ml of 5% HCl. After removal *in vacuo* of the reaction solvent, the residue was recrystallized in isopropanol to give white crystals, mp 135–137°, 0.296 g (67.8%); IR (KBr): 3275, 3075, 2930, 1725 (C=O), 1625 (C=O), 1525, 1220, 1050, 810, and 760 $cm^{-1}$; NMR ($CDCl_3$): δ 7.36 (8H, s, ArH), 5.46 (1H, s, CH), 4.14 (2H, d, N—$CH_2$), and 3.78 (3H, s, $OCH_3$); mass spectroscopy (70 ev): *m/e* (relative intensity) 318 (26) M + 2 − Cl, 317 (17), 316 (71) $M^+$ − Cl, 237 (36), 236 (24), 235 (55) $M^+$ − $C_4H_6O_3N$, 203 (12), 201 (43), 199 (29), 165 (71), 116 (69), and 88 (100).

*Anal.*—Calc. for $C_{17}H_{15}Cl_2NO_3$: C, 57.97; H, 4.29; N, 3.98. Found: C, 57.69; H, 4.40; N, 4.00.

**Urine Extraction**—Urine collected over 24 hr was pooled, and approximately 800-ml aliquots of this urine were adjusted to pH 2 with 10% HCl and continuously extracted with ether for 48 hr. The ether layer was reduced to 150 ml and extracted with 4 × 100 ml of 5% $NaHCO_3$. The sodium bicarbonate layer was acidified with 10% HCl and extracted with 4 × 200 ml ether. The ether phase was dried over sodium sulfate and treated with diazomethane followed by solvent evaporation. The resulting residue was taken up in 1 ml chloroform and used for GLC–mass spectroscopy.

The ether layer remaining after sodium bicarbonate extraction was reextracted with 4 × 100 ml of 5% NaOH. The sodium hydroxide extract was treated as the sodium bicarbonate extract above. The ether layer remaining after sodium hydroxide extraction was dried, methylated, and reduced to a residue which was taken up in 1 ml of chloroform. This procedure is outlined in Scheme I.

**Quantitation of Metabolites**—A known amount (25–50 mg/100 ml of urine) of bis(*p*-chlorophenyl)acetic acid (*p,p'*-DDA)[8] was added as an internal standard to 100–800-ml aliquots of urine. The urine was extracted and the methylated sodium bicarbonate fraction was prepared as already described. Standard solutions (5–10 mg/ml chloroform) of synthetic IV, VIII, XII, XIV, and the methyl ester of the internal standard were used for calibration. The 4-methoxy (VIII) and 3-methoxy (XI) compounds cochromatographed and gave an equal response of peak height *versus* amount injected. Synthetic VIII was used to quantitate the mixture of the two. The 4,5-dimethoxy synthetic standard (XII) was used to quantitate the 3,4-dimethoxy metabolite (XIII) because only XII was available as a crystalline compound of known purity. It is assumed that detector response *versus* amount injected is the same for both compounds. Amounts of standard solution were injected such that peak heights of the standard and the compound in urine extract were similar. Within the range of amounts injected, the graph of peak height *versus* amount injected was linear. GLC conditions, using an OV-1 column, are given in Table I.

The amounts of metabolites found in urine extracts were corrected by multiplying the ratio of the internal standard added to unextracted urine to the amount detected by GLC in urine extract. Values of milligrams metabolite extracted per milligram creatinine present were calculated. Creatinine determinations were by the method of Taussky (24). Creatinine and milligram metabolite values used are averages of three determinations. The percent daily dose excreted was calculated by dividing the milligrams metabolite excreted in a 24-hr urine sample by the dose in milligrams given on that day.

**Hydrolysis**—The internal standard bis(*p*-chlorophenyl)acetic acid was added to patient urine, which was then hydrolyzed by refluxing with an equal volume of concentrated hydrochloric acid for 4 hr. The hydrolyzed urine was extracted and a methylated sodium bicarbonate fraction was obtained and quantitated by the methods already described.

**Methylation**—Ethereal diazomethane was prepared by literature methods (25). Excess diazomethane was added to the sample dissolved in methanol and ether (1:1) and allowed to stand for 24 hr, analogous to literature procedures (12).

[8] Aldrich.

## REFERENCES


(1) M. M. Hart and J. A. Straw, *Biochem. Pharmacol.*, **20**, 1679(1971).

(2) T. Verdon, J. Bruton, R. H. Herman, and W. R. Beisel, *Clin. Res.*, **8**, 379(1960).

(3) L. Hellman, E. D. Weitzman, H. Roffwang, D. K. Fukushima, K. Yoshida, B. Zurmoff, and T. F. Gallagher, *J. Clin. Endocrinol.*, **31**, 227(1970).

(4) R. Gaunt, J. J. Chart, and A. A. Renzi, *Ergebn. Physiol.*, **56**, 115(1965).

(5) H. J. Grady, D. L. Azarnoff, R. Creager, D. H. Huffman, and J. Nichols, *Proc. Soc. Exp. Biol. Med.*, **119**, 238(1965).

(6) M. M. Hart, R. L. Reagen, and R. H. Adamson, *Toxicol. Appl. Pharmacol.*, **24**, 101(1973).

(7) J. Nichols, in "The Adrenal Cortex," H. D. Moon, Ed., P. B. Hoebner, New York, N.Y., 1961, p. 84.

(8) J. K. Faulkner and D. Woodcock, *J. Chem. Soc.*, **1961**, 5397.

(9) J. E. Sinsheimer, J. Guilford, L. J. Bobrin, and D. E. Schteingart, *J. Pharm. Sci.*, **61**, 314(1972).

(10) S. J. Cristol and H. L. Haller, *J. Amer. Chem. Soc.*, **67**, 2222(1945).

(11) "Organic Synthesis," vol. 1, A. H. Blatt, Ed., Wiley, New York, N.Y., 1941, p. 336.

(12) C. M. Williams and C. C. Sweeley, in "Biomedical Applications of Gas Chromatography," H. A. Szymanski, Ed., Plenum, New York, N.Y., 1964, p. 232.

(13) V. J. Feil, C. H. Lamoureux, E. Styrvoky, R. G. Zaylski, E. J. Thacker, and G. M. Holman, *J. Agr. Food Chem.*, **21**, 1072(1973).

(14) J. E. Sinsheimer and R. V. Smith, *Biochem. J.*, **111**, 35(1969).

(15) R. T. Williams, in "Biochemistry of Phenolic Compounds," J. B. Harbourne, Ed., Academic, New York, N.Y., 1963, p. 216.

(16) J. E. Peterson and W. H. Robison, *Toxicol. Appl. Pharmacol.*, **6**, 321(1964).

(17) J. D. Pinto, M. N. Camien, and M. S. Dun, *J. Biol. Chem.*, **240**, 2148(1965).

(18) L. Wallcane, R. Gingell, and S. Bronczyk, *Toxicol. Appl.*

*Pharmacol.*, **25**, 472(1973).
(19) R. H. Moy, *J. Lab. Clin. Med.*, **58**, 296(1961).
(20) T. Inoi, P. Gericke, and W. J. Horton, *J. Amer. Chem. Soc.*, **27**, 4599(1962).
(21) C. Postmus, Jr., I. A. Kaye, C. A. Craig, and R. S. Matthews, *J. Org. Chem.*, **29**, 2698(1964).
(22) "Organic Synthesis," vol. 1, A. H. Blatt, Ed., Wiley, New York, N.Y., 1941, p. 537.
(23) B. Belleau and G. Malek, *J. Amer. Chem. Soc.*, **90**, 1651(1968).
(24) H. H. Taussky, *J. Biol. Chem.*, **208**, 853(1954).
(25) "Organic Synthesis," vol. 2, A. H. Blatt, Ed., Wiley, New York, N.Y., 1943, p. 166.

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# Phosphorus–Nitrogen Compounds XVIII: Hydrazides and Thiosemicarbazides

**L. A. CATES[x] and T. L. LEMKE**

**Abstract** □ Phenylphosphono(thio)dihydrazides and phosphorylated thiosemicarbazides were synthesized for anticancer testing, and some of these agents displayed chelation properties. Reactions involving methylhydrazine resulted in substitution of $N^1$-protons. A study of diphenylphosphoro- and phosphoramidomethylhydrazides also indicated that the $N^1$-phosphorylated isomers are predominately formed.

**Keyphrases** □ Phosphorus–nitrogen compounds—synthesis of hydrazides and thiosemicarbazides as potential anticancer agents □ Nitrogen–phosphorus compounds—synthesis of hydrazides and thiosemicarbazides as potential anticancer agents □ Thiosemicarbazides, phosphorylated—synthesis as potential anticancer agents □ Hydrazides—synthesis of phenylphosphono(thio)dihydrazides as potential anticancer agents □ Anticancer agents, potential—synthesis of phenylphosphono(thio)dihydrazides and phosphorylated thiosemicarbazides

The hydrazino moiety frequently appears in agents possessing antitumor activity. The presence of this grouping in some of these compounds, such as procarbazine, indole-3-propionic acid hydrazide, α-hydrazino-ω-cyclohexylbutyric acid, 5-hydrazide 1-glutamic acid, and 2-(4-nitroso-7-oxo-1,3,5-cycloheptatrien-1-yl)isonicotinic acid hydrazide (1), is obvious, while in other agents its occurrence is less conspicuous. Various thiadiazole, pyrazole, triazeno, and azapurine oncolytics also contain the N—N bond. In a few cases, such as procarbazine and heterocyclic 2-carboxaldehyde thiosemicarbazones, the hydrazino grouping is believed to be involved in the cytotoxic process *via* redox reactivity (2) or chelation (3), respectively. Even hydrazine sulfate inhibits Walker carcinosarcoma by 28–94%, possibly through gluconeogenesis interference (4).

The confirmed activity of 4,4′,4″-phosphinylidyne trisemicarbazide[1] (5) in Walker carcinosarcoma encouraged the preparation of additional hydrazine compounds (I–XII) as potential antitumor agents. Six of the products can be classified as thiosemicarbazides, including a derivative (XII) of the anticancer agent 2-amino-1,3,4-thiadiazole, which contains a comparable structure in cyclic form. The remaining compounds are hydrazides (I–V and XI), including one containing a urethan portion (V) and a water-soluble dihydrazide (XI). This latter product may find interest in Walker carcinosarcoma in view of the probable greater sensitivity of this tumor toward hydrophilic drugs (6), while the methylhydrazides, I and II, were considered of interest in view of the established activity of procarbazine.

## DISCUSSION

During this investigation, particular attention was directed toward the reaction involving the monophosphorylation of methylhydrazine. This reactant possesses two nitrogens with replaceable hydrogens, and both $N^1$- or $N^2$-substitution products are possible (Scheme I). Theoretically, the more nucleophilic $N^1$ should preferentially undergo substitution. Debo (7), however, reported that $N^2$-phosphorylation occurred when phosphorochloridates were reacted with methylhydrazine in sodium carbonate solution. Support for this assignment is found in work with the reactions of methylhydrazine with esters and anhydrides of carboxylic acids (8). This study showed that, while anhydrides form mainly 1-acyl-1-methylhydrazides, esters yield chiefly 1-acyl-2-methylhydrazides, with the percentage of these latter isomers increasing with the size of the acyl group of the esters.

Debo's synthesis using diphenylphosphorochloridate was repeated during this study and the reaction products were examined by NMR spectroscopy. Of the three methylhydrazides reported by

[1] This agent has subsequently been found to possess a low therapeutic index.