Metabolic Fate of \( p', p''\)-DDT [1,1,1-Trichloro-2,2-bis\((p\text{-chlorophenyl})\)ethane] in Rats*

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The metabolic fate of \( p', p''\)-DDT\(^{*}\) (the principal and most potent isomer in commercial DDT) in mammals has been of considerable interest because this substance is widely used as an agricultural insecticide, and significant amounts of it are retained as residues in various farm and dairy products (1, 2) and are ultimately consumed by man. Prior to 1955, only DDT, DDE, and DDA had been identified as relevant compounds in mammalian urine and feces after ingestion of DDT (3). More recently, Jensen et al. (4) investigated the fecal metabolites produced in rats after oral administration of \( ^{14}\)C-labeled DDT and presented data suggesting that the major fecal products consist of DDA conjugated with either cholic acid or amino acids. DDA and DDE were not isolated as crystalline products; however, their presence in the feces was inferred (4) from the behavior of these compounds on a modified Mosbach column (5) and on a modified Mosbach (6) partition column. Only one metabolite of \( p', p''\)-DDT, namely \( p', p''\)-DDA, has been identified in the urine of rabbits, rats, and man (7-9), although the presence of two unidentified metabolites has been reported in the urine of rabbits which have ingested \( p', p''\)-DDT (7). It was suggested (7) that the unidentified metabolites were conjugates of \( p', p''\)-DDA, since nitration of either \( p', p''\)-DDA or either of the metabolites yielded an identical teta-nitrodichlorobenzophenone.\(^{2}\) The neutral product, DDD, has been identified as a DDT metabolite in the body fat of rats which have ingested \( p', p''\)-DDT (10).

The present study was undertaken to gain additional information concerning the identity of DDT metabolites produced in the rat. Fractionation of urine and feces from rats fed \( p', p''\)-DDT yielded crystalline \( p', p''\)-DDA and gave evidence for the presence of several amino acid conjugates of \( p', p''\)-DDA. Fractionation of feces from rats fed \( p', p''\)-DDA yielded a homogeneous acidic metabolite identified as a conjugate of \( p', p''\)-DDA with aspartic acid and serine, and produced evidence for the presence of four neutral metabolites, possibly identical with DBM, DBP, DBH, and DDE, respectively.

**EXPERIMENTAL PROCEDURE**

Preparations of \( p', p''\)-DDT and Related Compounds—\( p', p''\)-DDT was synthesized by treating trichloromethyl-\( p\text{-chlorophenyl}-\)carbinal prepared from chloroform and \( p\text{-chlorobenzaldehyde} \) with chlorobenzene essentially as described by Haller et al. (13). \( p', p''\)-DDE was prepared by dehydrochlorination of \( p', p''\)-DDT (13). \( p', p''\)-DDA was obtained by saponification of \( p', p''\)-DDE (15). \( p', p''\)-DBP was synthesized by cleavage of \( p', p''\)-DDE with chromic anhydride in acetic acid (13). \( p', p''\)-DBH was prepared by reduction of \( p', p''\)-DBP with zinc (16), and \( p', p''\)-DBM was prepared by decarboxylation of \( p', p''\)-DDA with potassium hydroxide in ethylene glycol (13). Properties and analyses of these products are given in Table I.

**Experimental Animals**—Male and female Wistar strain rats were fed Purina chow pellets with water ad libitum and main-

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\(^{*}\) Inquiries regarding this paper should be addressed to M. S. Dunn, University of California, Los Angeles. The experimental data are from a dissertation submitted by Joseph D. Pinto to the Graduate School of the University of California, Los Angeles, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The chemical names and structures for DDT and its derivatives, referred to by key letter designations in the text, are shown in Scheme 1. This work was supported by Grants GM-05574, B-1665, and AM 08523.01 from the United States Public Health Service.

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\(^{1}\) The abbreviations used are: DDT or \( p', p''\)-DDT, 1,1,1-trichloro-2,2-bis\((p\text{-chlorophenyl})\)ethane; DDE or \( p', p''\)-DDE, 1,1-dichloro-2-bis\((p\text{-chlorophenyl})\)ethylene; DDA or \( p', p''\)-DDA, bis\((p\text{-chlorophenyl})\)acetic acid; DBM, \( p', p''\)-dichlorodiphenylmethane; DBP, \( p', p''\)-dichlorobenzophenone; DBH, \( p', p''\)-dichlorobenzhydrol; TNB, tetranitrobenezophenone.

\(^{2}\) Analyses of this product were not presented (7), and it seems likely that its identification as tetranitrodichlorobenzophenone was not verified, since the present authors obtained a chlorine-free product, presumably to be tetranitrobenezophenone (TNB, Table I), by nitration of \( p', p''\)-DDA under similar experimental conditions.
tained in individual metabolism cages for collections of urine and feces separately. Each animal was lightly anesthetized with ether to facilitate administration of a daily dose of either p,p'-DDT or p,p'-DDA by gavage. The DDT was introduced at 10 mg per day as a 10-mg per ml solution in corn oil, and the DDA was introduced at either 25 or 100 mg per day as a 20-mg per ml solution in distilled water containing the amount of sodium hydroxide required to dissolve the DDA and yield a final pH of approximately 7.0. DDT derivatives (metabolites) in the urine and feces were detected and measured by the Schechter-Haller assay procedure (1). DDT was measured by the more specific Stiff-Costillo method (17,18).

**Concentration of Urinary Metabolites—** Urine samples were collected under toluene (a preservative) and stored at -15°. In a typical preparation, 5 liters of urine were concentrated to 50 ml by evaporation under reduced pressure at 40-45°. Celite 545 (50 g) and glacial acetic acid (50 ml) were mixed with the 50 ml of concentrated urine, and the mixture was agitated for 1 hour on a mechanical shaker and filtered. The filtrate was subjected to two additional treatments with Celite and discarded. The combined lots of Celite were extracted with methanol, and the extract was evaporated to dryness in a vacuum at 40-45°. Essentially all measurable DDT metabolites in the untreated urine were recovered in the residue left by evaporation of the methanol extract, and this residue was employed in the fractionation experiments to be described. Control urines, collected from animals not having been administered either DDT or DDA, did not contain detectable DDT metabolites.

**Concentration of Fecal Metabolites—** Feces samples were collected daily, dried for 1 hour under an infrared lamp, pulverized in a ball mill, and stored at -15° under acetone containing 5% glacial acetic acid. In a typical preparation 5 kg of feces were heated under reflux with 5 liters of 5% acetic acid in acetone at approximately 70° for 24 hours. The mixture was filtered, the residue was subjected to three additional extractions by the same procedure, and the final residue, containing practically no metabolites, was discarded. The combined acetone-acetic acid extracts were freed of solvents by evaporation.

**Fractionation Procedures—** Initial fractionations were effected either by simple partitions of the material between solvents or by countercurrent distribution procedures, since relatively large amounts of material could be handled in the latter way and preliminary experiments had indicated that hypothetical DDT metabolites could be separated readily in this manner, as shown in Fig. 1. Preliminary bulk fractionations were carried out in a 10-cell apparatus with a capacity of 250 ml of each liquid phase per cell. Fractionations of higher resolution were effected in either 100- or 400-cell instruments containing 10 ml of each phase per cell. Final purifications of the products were effected by column chromatography on silica gel and partition chromatography with the modified Mosbach (6) column as described by Jensen et al. (4).

**RESULTS**

**Metabolites in Post-DDT Urine—** Peeled urine from 2 rats given 10 mg of p,p'-DDT per day for 10 days yielded a concentrate containing 1.15 g of metabolites calculated as DDA. Extracting a 1-liter solution of the concentrate in ethyl ether with 1 liter of 3 N sulfuric acid and two 500-ml portions of 5% sodium chloride solution removed extraneous material without removing appreciable metabolites. Essentially all metabolites were removed from the residual ether solution by extracting the latter with 1 liter of 0.5 N sodium hydroxide. When the aqueous-

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**Table I. Analyses of p,p'-DDT and related products**

The all of the products were prepared as p,p'-isomers and were homogeneous by chromatography in Systems A, B, and C of Mitchell (11), which were capable of separating p,p'-DDT from its isomers under the conditions employed.

<table>
<thead>
<tr>
<th>Product</th>
<th>Melting point</th>
<th>Empirical formula</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td>108°</td>
<td>C_{12}H_{10}Cl_{2}</td>
<td>61.66</td>
<td>61.52</td>
</tr>
<tr>
<td>DDE</td>
<td>88</td>
<td>C_{12}H_{10}Cl_{2}</td>
<td>62.17</td>
<td>62.21</td>
</tr>
<tr>
<td>DDA</td>
<td>165</td>
<td>C_{12}H_{10}Cl_{2}</td>
<td>59.81</td>
<td>59.77</td>
</tr>
<tr>
<td>TNB</td>
<td>90-92</td>
<td>C_{12}H_{10}N_{4}</td>
<td>43.10</td>
<td>42.98</td>
</tr>
<tr>
<td>DBP</td>
<td>148</td>
<td>C_{12}H_{10}Cl_{2}</td>
<td>62.17</td>
<td>62.31</td>
</tr>
<tr>
<td>DBH</td>
<td>92</td>
<td>C_{12}H_{10}Cl_{2}</td>
<td>61.55</td>
<td>61.52</td>
</tr>
<tr>
<td>DBM</td>
<td>55</td>
<td>C_{12}H_{10}Cl_{2}</td>
<td>65.77</td>
<td>65.83</td>
</tr>
</tbody>
</table>

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*DDT and related compounds form colored products when subjected to nitration followed by treatment with alcoholic potassium hydroxide. The color is measured photometrically at 540 nm in the Schechter-Haller assay (1) for DDT metabolites.

*This method consists of treating the assay samples with xanthylid-potassium hydroxide-pyridine reagent, which is relatively specific for DDT. The red color produced from DDT is measured photometrically at 520 nm in the Stiff-Costillo assay (17).

*A purified diatomaceous silica powder produced by Johns-Manville.
countercurrent distribution (large cell apparatus) of the concentrate produced the patterns shown in Fig. 3a. These were essentially the same as those produced by a 9-transfer countercurrent distribution of corresponding post-DDA fecal material (Fig. 3b). Extraction of a chloroform solution of the dried solutes from cells 2 to 5 (Fig. 3a) with aqueous 1 N ammonium hydroxide solution removed essentially all metabolites from the chloroform. Extraction of the ammonium hydroxide solution with three consecutive portions of n-heptane after acidification with hydrochloric acid to pH 1.5 transferred most of the metabolites to the organic solvent phase, while leaving the bulk of extraneous material in the aqueous acid phase. The metabolites were extracted from the n-heptane with 0.5 N sodium hydroxide, and from the latter solution, which was acidified with hydrochloric acid to pH 1.5, with chloroform. The chloroform solution was evaporated to dryness, and the residue was subjected to a 380-transfer countercurrent distribution to yield a single product, which appeared to be reasonably homogeneous from the distribution pattern but contained only 21% DDA by assay. It was purified further by column chromatography with silica gel and finally by partition chromatography with the modified Mosbach (6) column as described by Jensen et al. (4). Its final

Table II

<table>
<thead>
<tr>
<th>Product</th>
<th>Identity</th>
<th>Melting point</th>
<th>Neutralization equivalent</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p,p'-DDA</td>
<td>165-166°</td>
<td>284</td>
<td>C H N Cl</td>
</tr>
<tr>
<td>2</td>
<td>TNB*</td>
<td>90-92</td>
<td>42.98 2.22 15.88</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>p,p'-DDA</td>
<td>165</td>
<td>284</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>p,p'-DDA</td>
<td>165</td>
<td>59.81 3.50 25.26</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>TNB*</td>
<td>90-92</td>
<td>42.96 2.14 15.88</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>p,p'-DDA</td>
<td>165-166°</td>
<td>285</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>TNB*</td>
<td>90-92</td>
<td>59.47 3.03 25.55</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>p,p'DDA</td>
<td>165-166°</td>
<td>264</td>
<td></td>
</tr>
</tbody>
</table>

* The isolations are referred to by number in the text.
* Mixture with the corresponding synthetic product (Table I) did not depress the melting point of any of the isolated products.
* Infrared and ultraviolet spectra were recorded and shown to be identical with those of the corresponding synthetic product listed in Table I.
* Identity with the corresponding synthetic product was confirmed by paper chromatography with descending butanol and 3% aqueous ammonia (3:1) on Whatman No. 3 paper and with ascending methanol and water (5:1) on Whatman No. 1 paper.
* Visible spectrum of the product was recorded and shown to be identical in benzene-alcoholic potassium hydroxide as described for the Schechter-Haller assay (1) with that of the corresponding derivative listed in Table I.
* Not detected.
* Identity with the corresponding synthetic product was confirmed by paper chromatography as in footnote d and additionally on Whatman No. 1 paper with the descending phase of propanol-water-concentrated ammonia (10:1:1) and the descending phase of ethyl acetate-acetic acid-water (2:1:1).
* Partition ratio observed by countercurrent distribution in n-heptane, methanol, water, and acetic acid (20:7:11:2) was identical with that for the corresponding synthetic product, Table I.
elution pattern was the same as that described (4) for \( p,p'\)-DDA, and evaporation of the eluates showing peak metabolite concentrations yielded a crystalline product which, after two successive crystallizations from 90% ethanol, was indistinguishable from authentic \( p,p'\)-DDA. The purified isolate is listed as Product 4, Table II, with its properties and analyses. Nitrination of the purified product yielded the TNB listed as Product 5, Table II. The latter was indistinguishable from the TNB produced by nitrination of authentic \( p,p'\)-DDA.

Subjection of the material in cells 0 and 1 (Fig. 4) to a 90-transfer countercurrent distribution yielded the distribution pattern shown in Fig. 4a. At least six separate DDT metabolites were indicated by these data. None of these components was successfully isolated and characterized, however, since homogeneity of the fractions was doubtful. More reliable results were sought with material from rats receiving DDA-fed rats yielded essentially the same distribution pattern (Fig. 4b).

Metabolites in Post-DDA Feces—A concentrate prepared from the pooled feces of 20 rats which had ingested 25 mg of \( p,p'\)-DDA per day for 15 days was carried through the same procedures as the post-DDT fecal material. The resulting countercurrent distribution patterns are shown in Figs. 3b and 4b. Evidently, the metabolites in the post-DDA feces are either similar to or identical with those in post-DDT feces (compare Figs. 3b and 4b with Figs. 3a and 4a). Several of the metabolites indicated by distribution peaks in Fig. 4b appeared to be conjugates of \( p,p'\)-DDA with amino acids, since nitrination of these fractions yielded TNB identical with that from authentic \( p,p'\)-DDA, and hydrolysis of the separated products (amino acid-free before hydrolysis) yielded from two to five amino acids identifiable by paper chromatography. These results were not conclusive, however, since homogeneity of the fractions was doubtful. More reliable results were sought with material from rats receiving larger quantities of \( p,p'\)-DDA.

Pooled feces from 25 rats given 100 mg of \( p,p'\)-DDA per day for 15 days yielded a concentrate containing 12.9 g of metabolites calculated as DDA. The concentrate was partitioned between chloroform and aqueous ammonia to yield a neutral fraction

![Graph](http://www.jbc.org/)

**Fig. 3.** Countercurrent distributions (9 transfers) of fecal products derived from rats given (a) \( p,p'\)-DDT, and (b) \( p,p'\)-DDA. A 10-cell apparatus (500 ml of total solvent capacity per cell) was employed with a solvent system consisting of equal parts of \( n \)-heptane and methanol. Absorbance values (left-hand vertical scale) from colorimetric assay of representative material are indicated by solid circles. Dry weight values (right-hand vertical scale) calculated for total cell contents are indicated by open circles. Values for cell zero are off scale for both absorbance (approximately 1.9) and dry weight (approximately 50).

**Fig. 4.** Countercurrent distributions (90 transfers) of relatively hydrophilic fecal products derived from rats given (a) \( p,p'\)-DDT, and (b) \( p,p'\)-DDA. A 100-cell apparatus was employed with a solvent system consisting of \( n \)-heptane, ethyl acetate, water, and acetic acid (3:1:4:1). Absorbance values (vertical scale) were derived from colorimetric assays of representative cell contents.

Isolation of \( p,p'\)-DDA from Hydrolysate of DDA Conjugate—A 50-mg sample of the DDA conjugate was hydrolyzed by heating it with 6 N hydrochloric acid under reflux for 18 hours in a 130° oil bath. Extraction of the hydrolysate with ether and evaporation of the ether extract to dryness left a crystalline residue, which was purified by crystallization from 80% ethanol to yield a product (Product 8, Table II) identifiable as \( p,p'\)-DDA.
Table III
Partition ratios of DDA conjugate before and after hydrolysis

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Partition ratio</th>
<th>DDA Conjugate</th>
<th>Synthetic p,p'-DDAb, c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before hydrolysis</td>
<td>After hydrolysis</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.00</td>
<td>0.66</td>
<td>0.68</td>
</tr>
<tr>
<td>II</td>
<td>0.11</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>III</td>
<td>0.85</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>IV</td>
<td>0.18</td>
<td>2.83</td>
<td>2.81</td>
</tr>
</tbody>
</table>

a System I: isooctane-95% ethanol (6:1); System II: n-heptane-methanol (1:1; system III: n-heptane-ethyl acetate-water-acetic acid (7:3:8:2); System IV: n-heptane-methanol-water-acetic acid (20:7:11:2).
b Measured as DDA by the Schechter-Haller assay (1). c Product described in Table I.

Table IV
$R_F$ values for aspartic acid, serine, and hydrolytic products of DDA conjugate

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>$R_F$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-Aspartic acid</td>
</tr>
<tr>
<td>I</td>
<td>0.18</td>
</tr>
<tr>
<td>II</td>
<td>0.08</td>
</tr>
<tr>
<td>III</td>
<td>0.22</td>
</tr>
<tr>
<td>IV</td>
<td>0.16</td>
</tr>
</tbody>
</table>

a System I: methanol-1-butanol-water (2:2:1); System II: 1-butanol-butane-water-cyclohexylamine (10:10:5:2); System III: 1-propanol-butane, water-diethylamine (10:10:5:2); System IV: 95% ethanol-concentrated ammonia-water (8:1:1).

Fig. 5. Countercurrent distribution (379 transfers) of neutral fecal products from rats given p,p'-DDA. A 400-cell apparatus was employed with the same solvents as in Fig. 1. Absorbance values (vertical scale) were derived from colorimetric assay of representative cell contents.

Observed partition ratios for the DDA conjugate before and after hydrolysis are listed in Table III. These data indicate large differences between the properties of the DDA conjugate and of free p,p'-DDA.

Demonstration of Aspartic Acid and Serine in the DDA Conjugate—A stock solution of the DDA conjugate was prepared in chloroform, and its concentration was precisely measured by assay. A sample corresponding to 2.80 μmoles of p,p'-DDA was freed of chloroform by evaporation, and the residual material was hydrolyzed by heating it with 3 N hydrochloric acid under reflux for 18 hours in a 130° oil bath. Extraction of the hydrolysate with ethyl ether removed the liberated p,p'-DDA (2.80 μmoles) quantitatively as indicated by assay of the extract.

The residual aqueous solution was evaporated to dryness, and the solids were dissolved in distilled water to a volume of 10 ml. Photometric ninhydrin analysis (18) of a portion of the solution indicated the presence of 5.70 μmoles of amino acids in the original hydrolysate. Chromatographic analysis (19, 20) of the remaining solution indicated the presence of 2.76 μmoles of aspartic acid and 2.80 μmoles of serine in the original hydrolysate. Ninhydrin-reacting components other than aspartic acid and serine were not detected either by the column chromatographic procedure or by paper chromatography. Observed $R_F$ values for the hydrolytic products are listed in Table IV.

Fractionation of Neutral Metabolites—A chloroform solution of the neutral fraction (that not extractable with aqueous alkali) from post-DDA feces was subjected to continuous liquid-liquid extraction with 10% acetic acid in water for 24 hours. This procedure extracted a large amount of extraneous material without removing any of the neutral metabolites. The residual chloroform solution was evaporated to dryness, and the residual solids, dissolved in nitromethane, were subjected to continuous liquid-liquid extraction with 20% ethyl acetate in n-heptane for 40 hours. This procedure carried practically all of the metabolites into the heptane-ethyl acetate mixture while leaving a large amount of extraneous material in the nitromethane phase. Evaporation of the heptane-ethyl acetate solution led to precipitation of a large amount of waxy material which proved to contain no significant amount of metabolites. Supernatant solution was separated from the waxy solid by decantation, and the solid was washed with n-heptane. The combined washings and supernatant solution were evaporated to dryness, and the residual material was subjected to a 380-transfer countercurrent distribution, which yielded the pattern shown in Fig. 5. Further studies of the four neutral metabolites indicated in Fig. 5 were, unfortunately, not feasible; however, it may be noted that their distribution pattern (Fig. 5) is somewhat similar to that produced by a mixture of the hypothetical metabolites, DBM, DBP, DBH, and DDE (Fig. 1). Possible identity of the neutral metabolites (Fig. 5) with DBM, DBP, DBH, and DDE, respectively, is not ruled out by lack of precise correspondence between the two distribution patterns (Figs. 1 and 5) since extraneous solutes in the fecal material would be expected to influence the properties of the solvent system sufficiently to effect appreciable changes in distribution ratios of the metabolites.

Discussion

DDT-resistant insects effect a detoxification of p,p'-DDT by converting it to the relatively nontoxic derivative, p,p'-DDE, as demonstrated in several laboratories (21-24). This detoxification, brought about by the dehydrochlorination of DDT, is catalyzed in the presence of glutathione by the enzyme DDT dehydrochlorinase (25-26). A similar detoxification step presumably occurs also in mammals since the fat of persons exposed to DDT has been demonstrated to contain more DDE than DDT (27, 28); however, for several mammals DDE is not markedly less toxic than DDT (9). Detoxification of DDT by formation of DDA in mammals (7-9) is presumably more effective than by formation of DDE, since DDA has a relatively low toxicity (29-30). In the present study, the administration of p,p'-DDA at 100 mg per day produced essentially the same symptoms of toxicity in rats as p,p'-DDT at 10 mg per day,
while \( p,p'-DDA \) at 25 mg per day produced no noticeable symptoms.

The toxic properties of DDT have generally been attributed in part to lipid solubility of the DDT molecule (31–34), and the lower toxicity of DDA might therefore be due to the relatively hydrophilic character imparted to DDA by its carboxyl function. Further reductions in toxicity are possibly effected when the hydrophilic properties of the molecule are enhanced by conjugation through the carboxyl group with relatively polar compounds such as amino acids, and substantial evidence for the formation of amino acid conjugates with \( p,p'-DDA \) has appeared in the present study.\(^a\) The one conjugate that was isolated as a homogeneous product yielded (upon acid hydrolysis) 45\% \( p,p'-DDA \), 17\% serine, and 21\% aspartic acid and had a minimum molecular weight (based upon DDA content) of 624.8. Calculated values, assuming 1 molecule each of serine and aspartic acid together with 8 molecules of water of hydration per molecule of \( p,p'-DDA \), are \( p,p'-DDA, 44.8\% \); serine, 16.8\%; aspartic acid, 21.2\%; and molecular weight, 627.4. It is of interest that the conjugate contained serine and aspartic acid, since serine has not previously been implicated as a detoxicant other than of xanthurenic acid in the rat (35), and aspartic acid has apparently not previously been reported in such reactions (36); however, asparaginase as well as glutamime is known to form derivatives with either phenylacetic acid or \( p \)-aminosalicylic acid in liver preparations (37). Similarly, glutamine forms a conjugate with phenylacetic acid in man (38) and the chimpanzee (39), and glycine conjugates with a variety of carboxylic and heterocyclic aromatic acids, \( \beta \)-substituted acrylic acids, and steroid acids (40). The conjugates of DDA formed from DDT in mammals are excreted principally in the bile (4, 41); however, the possibility of subsequent alteration of the fecal products by intestinal microorganisms is not excluded.

Biological conversion of \( p,p'-DDT \) to \( p,p'-DDA \) presumably proceeds through the intermediate compound, \( p,p'-DDE \) (42), although details of the steps from DDE to DDA are lacking. The processes by which \( p,p'-DDA \) is conjugated to amino acids likewise remain unknown; however, by analogy with hippuric acid biosynthesis (40), it might be expected that \( p,p'-DDA \) is first converted to dichlorophenylacetyl-CoA with the interaction of adenosine triphosphate, possibly by means of adenyldichlorodiphenylacetate, and then attached to the amino acid chain.

**SUMMARY**

Urinary and fecal products formed from \( p,p'-DDT (1,1\text{-trichloro-2,2-bis(p-chlorophenyl)ethane}) \) and \( p,p'-DDA (bis(p-chlorophenyl)acetic acid) \) administered to rats by gavage were investigated. \( p,p'-DDA \) was isolated as a crystalline product from both urine and feces of rats administered \( p,p'-DDT \). \( p,p'-DDA \) conjugated with other substances was demonstrated in the urine and feces of rats after administration of either \( p,p'-DDT \) or \( p,p'-DDA \). One such product was isolated in chromatographically pure form and demonstrated to be a conjugate of \( p,p'-DDA \), aspartic acid, and serine in equimolar proportions. Biological conversions of \( p,p'-DDT \) to \( p,p'-DDE \) (1,1-dichloro-2-bis(p-chlorophenyl)ethylene), free \( p,p'-DDE \), and conjugated \( p,p'-DDE \) are discussed in relation to DDT detoxification.

\(^a\) The products identified were obtained with relatively large amounts of administered substances and may not be the major products when much smaller doses are given.

**Acknowledgments**—Mount Sinai Hospital Division, Cedars of Lebanon-Mount Sinai Hospitals, Los Angeles, was the sponsoring institution for one of us (M. N. C.) during part of this study. Provision of facilities and assistance by the Hospital is gratefully acknowledged. The authors are indebted to Heather King for carrying out numerous microchemical analyses and to Donald Steele for infrared spectroscopy.

**REFERENCES**


5. **Modified Davidow column, Technical Development Laboratories, Technology Branch CDC, U. S. Public Health Service, Savannah, Georgia, Chemical Memorandum No. 1, 1st Revision, 1950.**


22. **Perry, A. S., and Hoskins, W. M., Science, 111, 60 (1950).**

23. **Sternberg, J., Kearns, C. W., and Bruce, W. N., J. Econ. Entomol., 43, 214 (1950).**

24. **Takahari, A. S., and Hoskins, W. M., J. Econ. Entomol., 46, 209, 829 (1953).**


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