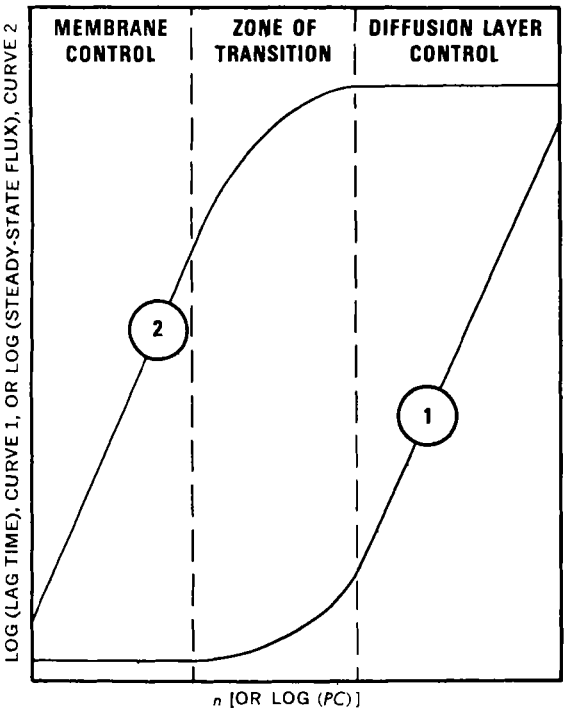

Figure 2—*Plot of the log (lag time), curve 1, or log (equimolar steady-state flux) against carbon number or its equivalent on the dimensionless axis, log* (PC). *If the two* Y*-axis variables are in compatible units, the limiting slope of 1 and the initial slope of 2 will be equal because the partition coefficient dependencies are the same in these regions.*

which will be recognized as being identical to Eq. 12. Thus, the principal condition is that $(PC)D_M\Sigma h_{AQ}$ must dominate the permeability expression. This will occur at some point in a homologous series because the partition coefficient will grow exponentially with chain length (8). Experimental verification will be presented in a subsequent report. For the present, it suffices to say that not only can one expect steady-state flux from equimolar solutions to level off within the series (3, 6), but one can also expect the time of barrier breakthrough to grow exponentially as chain length is increased once diffusion layer control has been attained. This dependency of lag time on chain length, n [or log (PC) which is directly related], is illustrated in Fig. 2. Also included in Fig. 2 for comparison is the relationship for concentration normalized steady-state flux which was previously derived (3). Both curves exhibit a marked change in slope at the crossover to diffusion layer control. The regions of total membrane control and total diffusion layer control are indicated, as is the transition zone between.

In 1963, Barrie *et al.* (9) derived steady-state and lag time equations for gaseous permeation of three-layer composite rubber membranes. The transient-state equation found in this paper can be converted so that it is applicable to the diffusion layer–membrane situation. This may be accomplished by multiplying each term in the equation (numerator and denominator) by S_2, the solubility in the middle layer of the laminate. The S_2/S_1 and S_2/S_3 ratios obtained are equivalent to $(PC)_{II}$ and $(PC)_{IV}$ as defined here. A careful examination of this equation in this form indicates that it reduces to Eq. 18 when $h_M \rightarrow 0$ and to Eq. 11 or 17 if the partition coefficient(s) is(are) large. Thus, when appropriate boundary conditions are placed on the Barrie *et al.* (9) expression, it is in total harmony with the equations derived independently in this report.

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Received September 7, 1971.
Accepted for publication December 1, 1971.
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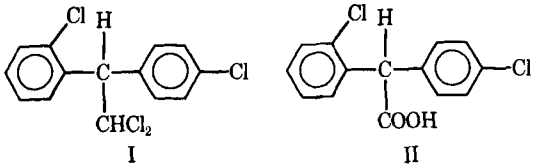
Identification of *o,p'*-Dichlorodiphenyl Acetic Acid as a Urinary Metabolite of 1-(*o*-Chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane

Keyphrases ☐ 1-(*o*-Chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane—metabolism, metabolite identification ☐ *o,p'*-Dichlorodiphenyldichloroethane—metabolism, metabolite identification ☐ Mitotane—metabolism, metabolite identification ☐ *o,p'*-Dichlorodiphenyl acetic acid—mitotane metabolite, isolation, characterization, GLC, TLC ☐ GLC—isolation, identification ☐ TLC—isolation, identification

Sir:

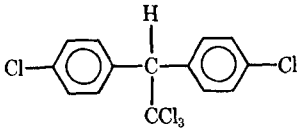
The recent FDA approval of mitotane, 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane (*o-p'*-dichlorodiphenyldichloroethane) (I), for treatment of adrenocortical carcinoma and adrenocortical hyperfunction (Cushing's syndrome) prompted this report of the isolation and characterization of *o,p'*-dichlorodiphenyl acetic acid (II) as a urinary metabolite of *o,p'*-dichlorodiphenyldichloroethane in rabbit and man. This result confirms Moy's (1) anticipation of *o,p'*-dichlorodiphenyl acetic acid as the principal metabolite based upon literature reports of the metabolism of the related compound, the insecticide *p,p'*-dichlorodiphenyltrichloroethane (III).

In initial experiments, four rabbits ingested over 11–18 days a total of 1.18–2.13 g. of Compound I, coated with the aid of hexane on food pellets. Results were



I

II



III

compared to control urine samples from the same rabbits previously fed hexane-treated pellets without drug. Urine was collected daily and frozen until extracted continuously with ether for 48 hr. The presence of II was indicated in the fraction containing strong acids[1]. This finding was made on the basis of flame-ionization detection by GLC of the bromosilyl ester compared to reference *o,p'*-dichlorodiphenyl acetic acid synthesized by the procedure of Cristol and Haller (2). The ester was prepared by the general procedure for bromosilyl esters of Bache *et al.* (3).

Chromatographic conditions were: 2% SE-30 on 80/100 Chromosorb W in a glass column 182.9 × 0.64 cm. (6 ft. × 0.25 in.); R_t 14 min. with helium at 60 ml. min.$^{-1}$; and injector, oven, and flame-ionization detector temperatures of 227, 202, and 217°, respectively.

The presence of II was also indicated by TLC comparison on 20 × 20-cm. plates to reference material using the system developed by Fehrimger and Ogger (4) for chlorinated pesticides on dipped microscope slides. Further modifications were the use of silica gel G and a polar solvent system (acetic acid–heptane, 1:4 by volume). Compound II (R_f 0.3) appeared as a characteristic dark-purple spot on the fluorescent background under UV light and as a red spot under white light.

The presence of II in the urine of three patients under treatment for adrenocortical carcinoma (3–16 g. of Compound I daily) was also indicated in a similar manner by GLC and TLC. Identification of II was from 17 l. of pooled urine from the same three patients with isolation by column chromatography (3 × 83 cm. of activated silicic acid) of the fraction containing the strong acids of the ether extracts of the pooled urine. Neither neutral metabolites nor Compound I were found in the urine following extraction by hexane or ether and monitoring by GLC and TLC. Half of the strong acid fraction (1.48 g.) dissolved in methanol was plated on silicic acid and transferred to the column. The chromatogram was eluated first with hexane and then with solvent mixtures containing increasing amounts of benzene in hexane[2]. Fractions (20 ml.) were monitored by GLC (flame- and electron-capture detection) and TLC.

No chlorinated metabolites were indicated until an estimated total of 0.5 g. of II was found in the 20% benzene–hexane fractions. Elution was continued by increasing polarity of solvent[2] through benzene, methanol, and acetic acid, but no other chlorinated metabolites were isolated. Fractions containing II were combined, reduced to dryness under vacuum, and recrystallized from 50% methanol to give a pale-yellow solid, m.p. 106–107° [lit. (3) m.p. 107–108°]. A mixed melting point showed no depression, and the isolated material had the same GLC and TLC characteristics and IR spectra as a reference sample of II synthesized by the literature procedure (2); $\lambda_{max.}^{methanol}$: 267 nm. (ε 500); $\lambda_{max.}^{KBr}$: 3000–2500 (COOH), 1710 (C=O), 1500 (aromatic), and 930 cm.$^{-1}$ (COOH dimer); mass spectrum[3] (70 ev.) *m/e* (relative intensity): 282 (27) M + 2, 281 (7) M + 1, 280 (40) M, 235 (100) M − COOH, 199 (30) M − (CO_2H and Cl), and 165 (70) M − (CO_2 and 2Cl); $[\alpha]_{365}^{34°}$ −11.25 (*c* 0.162 methanol).

The anilide derivative (5) was prepared and recrystallized from 50% ethanol to yield a white crystalline product, m.p. 200° [lit. (5) m.p. 202–203°]; $\lambda_{max.}^{methanol}$: 220 (ε 5400) and 245 nm. (ε 5000); $\lambda_{max.}^{KBr}$: 2950, 2875, 1675 (amide I), 1600, 1500 (aromatic), and 1550 cm.$^{-1}$ (amide II); mass spectrum (70 ev.) *m/e* (relative intensity): 357 (19) M + 2, 356 (8) M + 1, 355 (29) M, 320 (50) M − Cl, 235 (90) M − (—C(=O)—NH— ), 199 (75) M − (Cl and —C(=O)—NH— ), 165 (100) M − (2 Cl and —C(=O)—NH— ), 120 (70) —C(=O)—NH—  , and 77 (30) phenyl.

A mixed melting point and IR spectra indicated no difference between the anilide of isolated and reference Compound II.

An indication of the quantity of Compound II excreted in the urine of a typical adrenocortical carcinoma patient was obtained from an individual who had been receiving 4 g. of Compound I for 58 days. The patient had a blood level of 6 mcg./ml. of I as determined by the GLC procedure of Dale and Miles (6) for blood levels of the analogous insecticide, III. The patient excreted 8.3 mg. of II as the free acid in a 24-hr. sample of urine. Acid II was determined from a 25-ml. aliquot of a continuous ether extract (48 hr.) of a 425-ml. sample of acidified urine (pH 2). This determination was as the methyl ester prepared by the diazomethane procedure (7) for GLC assay of acids. An additional 39.7 mg. of acid II per 24-hr. sample of urine was indicated as arising from a conjugated form soluble in the continuous ether extract[4].

[1] The continuous ether extract (48 hr.) of the hydrochloric acid acidified urine (pH 2) was extracted with 5% $NaHCO_3$. The aqueous extracts were adjusted to pH 2 with hydrochloric acid and reextracted with ether.

[2] The solvent systems employed in the preparative column chromatography were 500-ml. portions of hexane and hexane in benzene mixtures (0.5, 1, and 2%) followed by 1-l. portions of hexane in benzene mixtures (5, 10, 20, and 50%); and 500-ml. volumes of benzene, ether in benzene (0.5, 1, 2, 5, 20, and 50%), ether, ethyl acetate, acetone, ethanol, methanol, 20% acetic acid in ethanol, and acetic acid.

[3] The authors are indebted to Dr. William L. Budde, Purdue Mass Spectrometry Center (U. S. Public Health Service Grant FR-00354), for obtaining the mass spectra on their Hitachi RMU6-D spectrometer.

[4] The residue from a 25-ml. aliquot of the ether extract was hydrolyzed (6 ml. of 20% hydrochloric acid for 4 hr.) and extracted with ether (3 × 10 ml.) before GLC determination as the methyl ester of II.

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Received March 29, 1971.
Accepted for publication November 19, 1971.
Supported in part by Institutional Research Grant IN-401 to the University of Michigan by the American Cancer Society.
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Methods for the Preparation of *trans*-3-Methyl-2-hexenoic Acid, the Malodorous Component of Schizophrenics' Sweat

Keyphrases □ *trans*-3-Methyl-2-hexenoic acid—schizophrenic sweat component, synthesis □ Schizophrenic sweat, malodorous component—synthesis of *trans*-3-methyl-2-hexenoic acid

Sir:

The malodorous component of schizophrenics' sweat (1) has been identified (2) as *trans*-3-methyl-2-hexenoic acid. Although recent studies failed to verify this observation, continued investigation was suggested (3). In the works cited, previously reported procedures (4, 5) were employed for the preparation of the ethyl ester of *trans*-3-methyl-2-hexenoic acid. Reinvestigation showed that these procedures yield four isomers of ethyl *trans*-3-methyl-2-hexenoic acid. The characterization of these isomers and development of alternate, more effective methods for preparing *trans*-3-methyl-2-hexenoic acid are described in this communication.

Table I—Products Formed with Methods A–D

Methods	Products	Ratio	Relative Retention Time[a]
A[b]	I*a*	1	0.59
	I*b*	2[c]	0.71
	II*a*	1[c]	0.71
	II*b*	2	1.00
B	II*c*	4	0.47
	II*d*	9	0.64
C	II*a*	2	0.69
	II*b*	3	1.00
D	II*a*	3	0.73
	II*b*	7	1.00

[a] Relative to ethyl *trans*-3-methyl-2-hexenoate (II*b*), retention time (from solvent front) = 5.1 min. (eight determinations). [b] The same ratio of products was obtained when $POCl_3$ or P_2O_5 dehydration was employed. [c] Proportions of these isomers were determined by NMR analysis.

Four procedures were used in preparing the ethyl and methyl esters of *trans*-3-methyl-2-hexenoic acid.

Method A—A recently reported (6) Reformatsky procedure was used to condense ethyl bromoacetate with 2-pentanone. A 67% yield of 3-methyl-3-hydroxyhexanoate was obtained after workup and distillation (109–112°, 10 mm.). Dehydration was accomplished with $POCl_3$ or P_2O_5 using the procedure of Kon and Nargund (5); distillation (90–97°, 10 mm.) gave a mixture of ethyl *cis*-3-methyl-3-hexenoate (I*a*), ethyl *trans*-3-methyl-3-hexenoate (I*b*), ethyl *cis*-3-methyl-2-hexenoate (II*a*), and ethyl *trans*-3-methyl-2-hexenoate (II*b*) in 69% yield.

Method B—Hydrogenation (5% palladium-on-charcoal) and hydrolysis (5% KOH in ethanol) of the unsaturated ester mixture produced by Method A gave an 85% yield of 3-methylhexanoic acid which was homogeneous upon GC[1]. By using a general procedure (7) for α-bromination, dehydrohalogenation, and esterification, 3-methylhexanoic acid was converted to a mixture of methyl *cis*-3-methyl-2-hexenoate (II*c*) and methyl *trans*-3-methyl-2-hexenoate (II*d*) in 33% yield.

Method C—The procedure of Wadsworth and Emmons (8) was used to condense triethyl phosphonoacetate with 2-pentanone to form II*a* and II*b* in 55% yield.

Method D—Reaction of (carbethoxymethylene)-triphenylphosphorane with 2-pentanone, using benzoic acid as a catalyst (9), gave an 87% yield of II*a* and II*b*.

Ester mixtures were submitted to preparative GC[2]. Compounds II*b* and II*d* were hydrolyzed (5% KOH in ethanol) to form *trans*-3-methyl-2-hexenoic acid in 80–86% yield. *trans*-3-Methyl-2-hexenoic acid had a melting point of 20° (uncorrected), and its spectral characteristics agreed well with reported data (2).

Gas chromatograms[1] of ester mixtures resulting from Method A showed three peaks (Table I). Isolation by preparative GC[2] of the components represented

[1] Hewlett-Packard 5750B equipped with a flame-ionization detector and a 1.83-m. (6-ft.) × 6-mm. i.d. glass column packed with 3% OV-17 on Gas Chrom Q (100/120 mesh); operated at 84° with a flow rate of 120 ml./min. (He).
[2] Varian Aerograph A-700 fitted with a 6.09-m. (20-ft.) × 9-mm. i.d. aluminum column packed with 30% SE-30 on Gas Chrom P (45/60 mesh); operated at 90° with a flow rate of 150 ml./min. (He).