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METABOLISM OF DIETHYLHEXYL PHTHALATE BY RATS

ISOLATION AND CHARACTERIZATION OF THE URINARY METABOLITES

P. W. ALBRO, R. THOMAS AND L. FISHBEIN

National Institute of Environmental Health Sciences, National Institutes of Health, Public Health Service and Department of Health, Education and Welfare, Research Triangle Park, N.C. 27709 (U.S.A.)

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SUMMARY

The metabolites appearing in the urine of rats fed di(2-ethylhexyl)phthalate have been isolated by two procedures, thin-layer chromatography of the free metabolites, or thin-layer and gas–liquid chromatography after treatment with diazomethane. The metabolites were characterized by infrared spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry. The only metabolites found were those to be expected from ω- and $(\omega-1)$-oxidation of mono(2-ethylhexyl) phthalate without attack on the aromatic ring. Conjugates were apparently not formed, and free phthalic acid amounted to less than 3% of the urinary metabolites.

INTRODUCTION

A surge of concern about possible hazards of phthalate plasticizers has been set off by discovery of the leaching by blood of phthalates from storage bags in blood banks[1]. A recent conference on phthalate esters[2] repeatedly emphasized that very little is known about the metabolism of phthalates, although some work was presented[3] indicating that radioactivity associated with the carbonyl carbons of ^{14}C-labeled di(2-ethylhexyl) phthalate (DEHP, the most widely used phthalate plasticizer) administered to rats was eliminated rapidly in the urine and feces. On oral administration about 55% of the ^{14}C was eliminated in the urine and 35% in the feces, while on intravenous administration the distribution shifted more in favor of the urine[3].

We have confirmed the distribution studies reported at the phthalate ester conference relative to [^{14}C]DEHP in rats, and here report the chromatographic isolation and identification of the DEHP metabolites found in rat urine.

MATERIALS AND METHODS

Synthesis of DEHP and related compounds

Phthalic anhydride (7.40 g, Fisher Certified, >99.8%), 2-ethyl-1-hexanol

(65.0 ml, Eastman P3608) and *p*-toluenesulfonic acid (0.5 g, Fisher Certified) in 125 ml of toluene were refluxed for 7 h. The toluene solution was washed twice with 0.4 *M* K_2CO_3, after which the organic phase was concentrated (rotary evaporator) to 25 ml at 60°. Residual toluene and ethylhexanol were distilled off *in vacuo* (up to 180°, 100 mm Hg). Final purification was by passage through Florisil in hexane–diethyl ether (9:1). The product was at least 99.8% pure di(2-ethylhexyl) phthalate by gas–liquid chromatography (GLC) on OV-101 and thin-layer chromatography (TLC) on Silica Gel GF in petroleum ether–diethyl ether–acetic acid (87:13:1). The structure was confirmed by infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS).

Labeled DEHP was synthesized as above, reacting 4.0 mg of [7-^{14}C]phthalic anhydride (ICN Chemical & Radiochemical Div., 17.6 mCi/mmole) and 0.1 ml of 2-ethyl-1-hexanol in the presence of 1 mg of *p*-toluenesulfonic acid. The final product contained 85% of the starting ^{14}C and had a specific radioactivity of 45 μCi/mg, >99% of the ^{14}C chromatographed with DEHP on OV-101 and Silica Gel GF.

Di-*n*-octyl phthalate and dimethyl phthalate were synthesized by appropriate modifications of the above procedure. Mono(2-ethylhexyl) phthalate (MEHP) was synthesized by refluxing 148 mg of phthalic anhydride and 0.65 ml of 2-ethyl-1-hexanol in 25 ml of toluene for 7 h. The mono ester was extracted into 0.4 *M* K_2CO_3 and then released for extraction into diethyl ether by acidification with HCl. Monomethyl mono(2-ethylhexyl) phthalate was made by treating the monoester with diazomethane[4].

2-Ethylhexan-1,2-diol was synthesized by treating a 10% solution of 2-ethyl-1-hexene (Chemical Samples Co., >99%) in 85% formic acid with 30% hydrogen peroxide dropwise for 20 min at room temperature with constant stirring. The product was hydrolyzed with KOH to eliminate formate esters, partitioned between heptane and 90% methanol to remove most of the unreacted hydrocarbon and epoxide, and finally chromatographed on 5% hydrated Florisil. The diol was obtained in 10% yield in the diethyl ether–methanol (98:2) fraction.

Sources of reference compounds

2-Ethylhexanoic acid, 3-methyl-3-nonanol, 2-ethyl-1-hexene, and 2-ethyl-1,3-hexanediol were the highest purity materials available from Chemical Samples Co., Columbus, Ohio. Hydrocarbon standards for GLC retention index determinations were from Applied Science Laboratories, State College, Pa. Fatty acids, methyl esters, and methyl heptadecyl ketone were from LaChat Chemicals, Inc., Chicago Heights, Ill. Nonadecan-2-ol was made by reduction of the ketone with $NaBH_4$. These reference compounds were prepared to aid in interpreting the various spectra.

GLC and determination of retention indices

GLC apparatus and retention index (*R.I.*) measurements were as described previously[5]. The columns used were 3.25 mm O.D., stainless steel, 1 m and 3.5 m in length, packed with 10% OV-3 on 100–200 mesh Gas-Chrom Z. The two column lengths permitted all of the analytical work to be done at 180°, providing comparable *R.I.* values.

Preparative GLC was done on the Hewlett-Packard Model 5750, using a 6.5-mm O.D. stainless-steel column packed with 5% OV-1 on 80–100 mesh Gas-Chrom

Q. Fractions were trapped in sand-packed tubes as described earlier[6]. The trapped fractions were assayed for radioactivity using a BBOT cocktail[7] and a Packard Tri-Carb scintillation counter.

Spectroscopy and spectrometry

Both electron impact and chemical ionization mass spectra were obtained using a Finnigan 1015 S/L quadrupole mass spectrometer interfaced to a PDP 8/*e* "System 150" computer unit. Samples were introduced by elution from a 1.7-m glass column packed with 3% OV-1 on 100–120 mesh Gas-Chrom Q mounted in the Varian Aerograph Model 1400 gas chromatograph associated with this instrument. Electron impact spectra were obtained at ionization energies of 10, 20, and 70 eV. Chemical ionization spectra were obtained with methane and isobutane as reagent gases, both at pressures of 600 μ. Operating parameters were: emission current, 500 μA; electron energy, 10, 20, or 70 eV; lens, 14; repeller, 2; ion energy, 5; temperatures: manifold, 190°; ionizer, 75°; transfer line, 175°; interphase, 250°; power supply, 3000 V.

NMR spectra were taken in deuterochloroform solvent, with tetramethylsilane or chloroform as internal reference, using a Varian T-60 instrument. Those compounds insoluble in deuterochloroform (some of the free acids) were examined in deuteroacetone. Chemical shifts were measured in p.p.m. relative to tetramethylsilane $\equiv$ 0, or chloroform $\equiv$ 7.24.

IR spectra were taken both in carbon tetrachloride solution and as neat films on NaCl plates, using a Perkin-Elmer Model 621 spectrophotometer.

Isolation of DEHP metabolites

Adult (300–400 g) male CD rats (Charles River) were fed 0.2-ml doses of DEHP by gavage, and maintained on D&G Laboratory Diet in metabolism cages. In general, rats received two doses of DEHP 24 h apart, and urine was collected for 48 h after the first dose. The urine was collected in bottles containing a thymol crystal to inhibit bacterial action.

Urine from rats fed the ^{14}C-labeled DEHP was combined with the urine containing unlabeled metabolites to serve as marker during chromatography. The urine was diluted with an equal volume of water, adjusted to pH 2.0 with HCl, and extracted three times with volumes of diethyl ether equal to that of the diluted urine. The ether extracts were combined, washed once with 0.1 *N* HCl, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed at 40° using a rotary evaporator, after which the residue was dissolved in a small volume of diethyl ether for chromatography.

The extract was first streaked on 500 μ-thick plates of Adsorbosil-5 and developed in chloroform–methanol–acetic acid (143:7:2). The radioactive zones were located using a Varian Aerograph-Berthold LB 2723 radio scanner, scraped into sintered glass filters, and eluted with acetone–formic acid (20:1). The solvent was evaporated under nitrogen, and the material from each zone was dissolved in diethyl ether. Each fraction was individually streaked on 250-μ Silica Gel GF plates that had been pre-developed in acetone and reactivated at 110°. These plates were developed in lined tanks with 95% ethanol–water–conc. NH_4OH (100:12:16)[8]. The radioactive zones were again located using the radioscanner, outlined under UV light, scraped

and eluted. The solvent was evaporated under nitrogen with addition of small amounts of benzene to eliminate all of the formic acid.

This procedure gave a total of five radioactive fractions, all of which were found to be carboxylic acids on the basis of their IR spectra. Each fraction represented one of the five radioactive metabolites detectable when the total urine extract was treated with diazomethane and preparatively gas chromatographed on OV-1. The same products could be isolated by TLC separation of the diazomethane-treated urine extract in petroleum ether–diethyl ether–acetic acid (80:20:1), except that in the latter case two of the metabolites migrated together and required preparative GLC for separation. Each of the metabolites isolated as the free acids gave, when treated with diazomethane, only one radioactive peak (at least 94% radiochemically pure) when examined by preparative GLC on OV-1.

Preparation of derivatives

Methyl esters were prepared from the free acids by treatment with diazomethane in diethyl ether–methanol (9:1)[4]. Treatment under our conditions produced no reaction with methyl heptadecyl ketone or 3-methyl-3-nonanol, but phenols did react to give methyl ethers.

Esters were hydrolyzed by heating in closed tubes for 90 min in 50% aq. ethanol containing 2 *N* KOH. Products were extracted into diethyl ether after acidification to pH 1 with HCl and dilution to 25% ethanol.

Trimethylsilyl derivatives were prepared by heating for 15 min at 80° in pyridine–bis-trimethylsilyl trifluoroacetamide–trimethylchlorosilane (200:100:1). Derivatives of the lower-molecular-weight compounds were made by substituting methylene chloride for the pyridine.

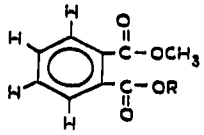
RESULTS AND DISCUSSION

It had previously been reported[3] that TLC of untreated urine from rats fed [^{14}C]DEHP and of the ether extract of acidified urine gave identical patterns of radioactivity. This implied that artifacts were not produced during extraction. We found that 75% of the radioactivity in urine of rats given [^{14}C]DEHP could be extracted into an equal volume of diethyl ether at pH 3. There was no change either in ^{14}C extracted or in TLC patterns on incubating with β-glucuronidase (Sigma Type I at pH 7.0), or aryl sulfatase (Sigma Type III at pH 4.5), suggesting the probable absence of glucuronide or sulfate conjugates. Acid hydrolysis (1 *N* HCl, 4 h, 100°) also failed to increase the amount of radioactivity extractable after readjusting to pH 3, suggesting the probable absence of glycine conjugates. None of the metabolites showed amide bands in the IR. Finally, >98% of the radioactivity present in the urine was extracted into three changes of either, at pH 2. These observations led us to conclude that the DEHP metabolites were excreted without conjugation.

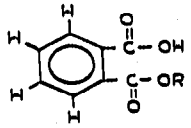
Alkaline hydrolysis of either the total ether extract or of the individual metabolites gave phthalic acid as the only detectable ^{14}C-labeled product. This was established by TLC of the products on Silica Gel GF plates in (a) 95% ethanol–water–conc. NH_4OH (100:12:16) and (b) benzene–methanol–acetic acid (90:16:8), in which free *o*-phthalic acid had R_F values of 0.28 and 0.84, respectively, and confirmed by radio-GLC of the diazomethane-treated hydrolysis products on OV-1 with authentic

dimethyl phthalate as carrier. Thus we concluded that the rats did not modify the phthalic moiety of DEHP in producing the urinary metabolites.

Electron impact mass spectra of the diazomethane-treated metabolites all showed *m/e* 163 as the base peak; this is the base peak for all the phthalates tested having a part structure:



whereas all the phthalates having both aliphatic moieties higher than methyl give *m/e* 149 as base peak[2]. Both NMR and IR spectra confirmed a 1,2-disubstituted benzene structure for the metabolites. However, only 2.8% of the radioactivity extracted from the urine migrated on TLC or GLC with phthalic acid or dimethyl phthalate, respectively. Thus the four major urinary metabolites of DEHP in rats have the part structure:



where R may be any group other than methyl. MEHP, however, was not present in the urine. A summary of TLC R_F values and GLC *R.I.* values for the free and derivatized DEHP metabolites is given in Table I.

TABLE I

CHROMATOGRAPHIC PARAMETERS FOR THE DEHP METABOLITES[a]

TLC R_F values obtained with developing systems: (A) chloroform–methanol–acetic acid (143:7:2); (B) 95% ethanol–water–conc. NH_4OH (100:12:16); (C) petroleum ether–diethyl ether–acetic acid (87:13:1).
GLC *R.I.* values determined on OV-3 at 180°; carrier gas, helium.

Compound	*Procedure*	*R_F/R.I. value*					
		1	*2*	*3*	*4*	*5*[b]	*MEHP*
Acid	TLC A	0.75	0.69	0.60	0.49	0.30	0.85
Acid	TLC B	0.87	0.75	0.65	0.63	0.28	0.91
Methyl ester	TLC C	0.25	0.07	0.49	0.49	0.75	0.80
Methyl ester	GLC	2261	2271	2382	2181	1508	2065
Ester-TMS[c]	GLC	NF[d]	2309	NF[d]	NF[d]	NF[d]	NF[d]
Percentage of ^{14}C		22	27	39	9	2.8	0

[a] Metabolites 1–5 were found in the urine.
[b] And *o*-phthalic acid (Analabs, Inc.).
[c] TMS = trimethylsilyl derivative.
[d] NF = none formed.

NMR spectra of the methyl esters of the four major DEHP metabolites are summarized in Table II. Metabolite 5 has been omitted in order to simplify the table;

TABLE II

NMR SPECTRA OF MAJOR DEHP METABOLITES[a]

δ (p.p.m.)	Class	Number of protons			
		1	2	3	4
~7.65	complex	4	4	4	4
4.24	doublet	2	2	2	2
3.90	singlet	3	3	3	3
3.64	singlet	–	–	3	3
2.40	triplet	2	–	–	–
2.30	triplet	–	–	2	2
2.12	singlet	3	–	–	–
2.09	broad	–	1	–	–
~1.7+	complex	3	4[b]	1	1
~1.4+	complex	2	4	6	2
1.23	doublet	–	3	–	–
0.95	triplet	3	3	3	3
	Total	22	24	24	20

[a] The methyl ester derivatives of metabolites 1–4 are analyzed.
[b] Drops to 3 when ^2H_2O is added.

it matched the available reference compound *o*-phthalic acid in IR, NMR, and mass spectra. The NMR spectra of the free acid metabolites differ from those described in Table II in lacking the methyl ester peaks and in showing –COOH protons above 9 p.p.m. Acid metabolites 1 and 2 showed one –COOH each, while metabolites 3 and 4 showed two.

TABLE III

PRINCIPAL FRAGMENTS IN THE ELECTRON IMPACT MASS SPECTRA OF THE DEHP METABOLITES[a]
Electron impact, 20 eV.

a.m.u.	Relative abundance (%)[b]			
	1	2	3	4
41	5.2	4.7	5.7	3.5
43	56.8	11.6	3.4	1.8
44	3.5	—	1.2	—
45	—	7.0	—	—
55	15.0	15.0	13.8	6.5
56	4.1	7.4	1.2	—
57	2.7	4.6	1.1	—
58	4.1	7.8	—	—
59	1.4	3.8	5.5	3.8
65	—	—	1.2	—
67	1.9	2.8	3.0	1.3
68	12.8	13.9	—	25.2
69	17.2	21.3	3.5	16.9
70	1.6	9.3	2.8	—
71	9.8	7.2	—	1.3
72	1.4	—	—	—
74	—	—	8.8	7.1
76	1.9	3.8	6.4	1.3
77	9.0	7.2	12.0	10.3
79	1.6	1.7	1.4	1.5

(Continued on p. 327)

TABLE III *(continued)*

a.m.u.	*Relative abundance*[b]			
	1	*2*	*3*	*4*
81	—	14.4	2.3	—
82	6.8	2.1	13.4	—
83	39.6	3.8	8.3	—
84	9.8	7.8	—	—
85	2.2	3.4	1.4	2.3
87	—	—	4.1	7.1
91	1.6	1.5	1.2	2.5
92	5.5	4.7	5.3	9.3
93	6.8	1.7	1.2	—
95	—	13.5	1.6	—
96	—	—	9.7	29.0
97	20.5	3.6	2.7	7.6
98	1.9	—	—	—
99	—	3.2	—	3.5
101	—	—	1.1	—
104	4.6	6.6	5.7	5.5
105	4.1	4.4	3.4	4.8
108	41.7	2.8	—	—
109	9.5	1.9	—	—
110	—	7.2	—	—
111	20.5	4.0	—	—
112	1.9	—	—	—
113	1.6	3.2	—	10.6
114	—	—	3.2	—
120	—	—	1.1	—
124	—	—	15.2	—
125	1.4	—	3.4	—
126	17.5	1.1	—	—
127	5.2	2.1	1.2	—
128	—	—	—	24.9
129	—	—	—	7.8
132	1.1	1.3	1.2	1.8
133	4.6	4.0	3.4	4.8
135	4.4	3.8	3.7	6.5
136	1.9	2.1	1.2	4.5
149	45.6	64.9	34.5	39.0
150	3.3	5.3	2.7	3.3
156	—	—	7.2	—
157	—	—	2.1	—
163	100.0	100.0	100.0	100.0
164	10.2	10.2	11.2	18.8
165	1.1	1.1	1.1	1.6
181	6.1	15.6	10.9	2.0
182	—	1.4	1.1	—

[a] Only peaks having relative abundance >1% are listed for metabolite esters 1–4.
[b] Relative to a.m.u. 163 = 100.0%.

Mass spectra are summarized in Table III (20 eV electron impact) and IV (methane chemical ionization). Electron impact spectra at 10 and 70 eV provided no information not present in the 20 eV spectra, and are not reported here. The isobutane chemical ionization spectra were less useful for structural identification than were the spectra obtained using methane; however, the isobutane-derived spectra did confirm the molecular weights of the metabolites and indicated which peaks in the methane-derived spectra were due to recombination.

TABLE IV

PRINCIPAL FRAGMENTS[a] IN THE METHANE CHEMICAL IONIZATION MASS SPECTRA OF THE DEHP METABOLITES

a.m.u.	*Relative abundance*[b]			
	1	*2*	*3*	*4*
61	—	—	6.4	—
69	—	5.3	6.3	—
71	—	—	38.3	—
83	—	—	—	—
85	—	—	35.0	—
99	—	—	67.1	—
100	—	—	6.3	—
108	2.2	—	—	—
109	1.2	—	—	—
111	—	28.6	—	—
112	—	2.6	—	—
127	100.0	7.8	—	—
128	23.0	—	—	1.9
129	1.8	31.0	—	100.0
130	—	2.4	—	8.1
149	1.0	14.6	2.5	—
155	1.2	—	—	—
156	—	—	1.6	—
157	—	1.1	87.5	—
158	—	—	8.1	—
163	38.9	100.0	100.0	49.2
164	4.1	10.5	8.0	5.8
165	—	1.4	—	—
177	—	2.6	—	—
181	2.1	70.4	11.0	2.0
182	—	6.2	—	—
191	—	1.3	—	—
197	—	—	1.7	—
209	—	1.6	1.6	—
221	—	1.4	—	—
269	—	—	1.7	—
291	—	9.9	—	—
292	—	1.8	—	—
305	—	—	4.8	—
307	5.6	—	—	—
308	1.2	—	—	—
309	—	18.2	—	2.8
310	—	3.3	—	0.5
335[c]	1.6	—	—	—
337[c]	—	1.8	28.7	1.8
338	—	—	4.8	—
365[c]	—	—	5.7	—

[a] Only fragments having relative abundance $\geq 1\%$ are listed for metabolite esters 1–4.
[b] Relative to a.m.u. 127, 163, 129 = 100.0% for metabolites 1, 2 and 3, and 4, respectively.
[c] Recombination fragments.

The NMR spectra were interpreted as follows: The peak complex around 7.65 p.p.m. was identical to that given by all of the reference phthalates and is assigned to the four adjacent aromatic protons. The singlet at 3.9 p.p.m. was assigned to a methyl ester of a benzoic acid, while that at 3.64 p.p.m. suggested the methyl ester of an aliphatic acid. The triplet at 2.4 p.p.m. for ester 1 reflects a $-CH_2-CH_2-\overset{|}{C}=O$, and

in concert with a 3-proton singlet at 2.12 p.p.m. indicates a methyl ketone. The 2-proton triplets at 2.3 p.p.m. were assigned to $-CH_2-COOCH_3$ structures. The proton at 2.09 p.p.m., along with an hydroxyl proton at ~1.8 p.p.m. in metabolite 2 were assigned to a C–CHOH–C unit, and this was confirmed by the observation that metabolite 2 was the only one of the five metabolites giving a trimethylsilyl derivative. The 3-proton doublet at 1.23 p.p.m. in the spectrum for metabolite 2 then could be assigned to the structure $CH_3-CHOH-C$. The groupings at 0.95, ~1.4, and ~1.7 p.p.m. were then assigned to CH_3, CH_3, and CH groups, respectively, attached to saturated carbon.

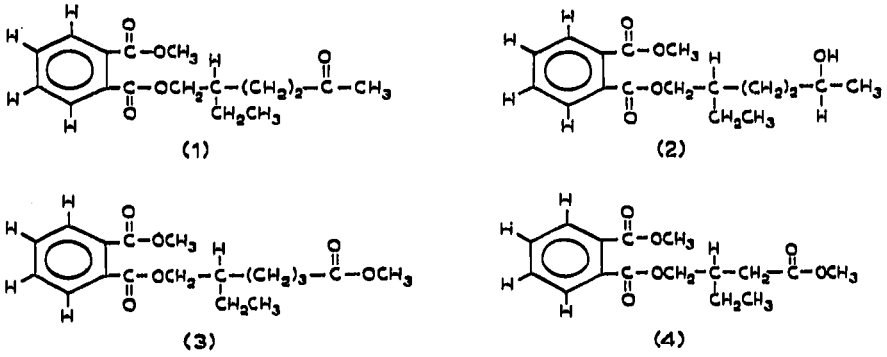


Fig. 1. Proposed structures for the major DEHP metabolites after methyl esterification.

The NMR spectra were then consistent with the structures given in Fig. 1 for the major DEHP metabolites. The molecular weights of the four esterified metabolites as determined from the chemical ionization mass spectra, 306, 308, 336, and 308, for esters 1–4, respectively, are also consistent with the structures given in Fig. 1.

Ester 1 fragments to give a major peak in the mass spectrum *m/e* 43, which is attributed to $(CH_3-C\equiv O)^+$. Cleavage of the side chain at oxygen and again at the carbonyl gives, with loss of one hydride ion, the major fragment at *m/e* 83. The large fragment at *m/e* 126, correlating with the base peak of 127 in the chemical ionization spectrum, derives from the complete side chain cleaved at oxygen with or without loss of a hydride ion. This fragment, with rearrangement and loss of water can give the major fragment *m/e* 108, while loss instead of the ethyl branch gives *m/e* 97, loss of methyl 111. *m/e* 149 is assigned to protonated phthalic anhydride, while *m/e* 163 results from cleaving the longer side chain from methyl phthalates.

Ester 2 shows the expected loss of water to give *m/e* 291 in the chemical ionization spectrum. Having already lost water, several of the major fragments (*m/e* 81, 95) occur two a.m.u. below the corresponding fragments from ester 1. Where water is not previously lost, giving rise to *m/e* 129 as the intact side chain cleaved at oxygen, subsequent loss of water yields the conspicuous fragment at *m/e* 111 in the chemical ionization spectrum.

Ester 3 shows the *m/e* 74 fragment typical of aliphatic methyl esters (as does ester 4). The intact side chain is here represented in the chemical ionization spectrum at *m/e* 157. Cleavage at carbonyl gives a residual side chain fragment at *m/e* 99, which shows loss of subsequent $-CH_2-$ groups to give *m/e* 85, and 71. In the electron

impact spectrum, the intact side chain (less one hydride ion) appears conspicuously at *m/e* 156, losing methanol to give *m/e* 124.

Ester 4 shows the intact side chain as *m/e* 129 (the base peak in this case) in its chemical ionization spectrum. This corresponds to *m/e* 128 in the electron impact spectrum (again, loss of hydride), loss of methanol giving *m/e* 96 and loss of "methyl formate" giving *m/e* 68. Thus the mass spectra were entirely in agreement with the structures postulated in Fig. 1.

The GLC *R.I.* values listed in Table I are also in agreement with the structures postulated in Fig. 1. Ester 4 appears 200 *R.I.* units less than ester 3, in agreement with it being homologous but two carbons shorter. The similarity in *R.I.* for ester 1 and 2 is in agreement with the similar affinities of OV-3 for secondary alcohols and the corresponding ketones (*R.I.*, methyl heptadecyl ketone = 2118, nonadecan-2-ol = 2120).

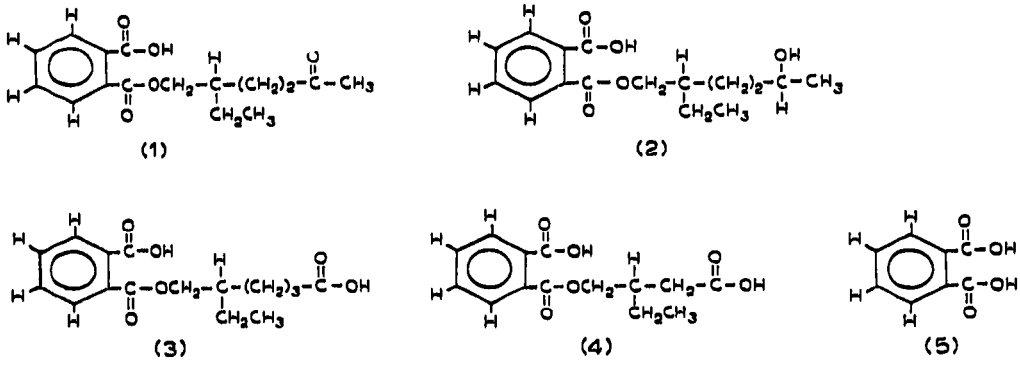


Fig. 2. Metabolites of DEHP as excreted in the urine of rats.

The original metabolites, isolated as the free acids, thus had the structures shown in Fig. 2. It appears most probable that DEHP is first hydrolyzed to MEHP, which then undergoes ω-oxidation and (ω—1)-oxidation[9], probably in the liver. The alcohol intermediates may then be oxidized to the level of ketone after (ω—1)-oxidation[10] or acid after ω-oxidation[9,11]. The acid metabolite 3 may then undergo one round of β-oxidation to yield metabolite 4. The metabolites found in the urine thus suggest that mono(2-ethylhexyl) phthalate is handled like a fatty acid in the rat. α- and β-oxidation are initially impossible, so ω-oxidation results.

REFERENCES

1 R. J. JAEGER AND R. J. RUBIN, *Science*, 170 (1970) 460.
2 *Phthalic Acid Esters Conference, N.I.E.H.S., Pinehurst, N.C., September 6–7th, 1972.*
3 C. O. SCHULZ AND R. J. RUBIN, *Phthalic Acid Esters Conference, N.I.E.H.S., Pinehurst, N.C., September 7th, 1972.*
4 TH. J. DEBOER AND H. J. BACKER, *Rec. Trav. Chim.*, 73 (1954) 229.
5 P. W. ALBRO AND L. FISHBEIN, *J. Chromatogr.*, 69 (1972) 273.
6 P. W. ALBRO AND J. C. DITTMER, *Biochemistry*, 8 (1969) 394.
7 P. W. ALBRO AND L. FISHBEIN, *Biochim. Biophys. Acta*, 219 (1970) 437.
8 D. BRAUN AND H. GEENEN, *J. Chromatogr.*, 7 (1972) 56.
9 K. WAKABAYASHI AND N. SHIMAZONO, *Biochim. Biophys. Acta*, 70 (1963) 132.
10 I. BJÖRKHEM AND M. HAMBERG, *J. Biol. Chem.*, 246 (1971) 7417.
11 M. HAMBERG AND I. BJÖRKHEM, *J. Biol. Chem.*, 246 (1971) 7411.