3-Phenylindole (XVI)——Phenylacetaldehyde phenylhydrazone (m.p. 59～60.5°; 1.3 g.) (lit.,[37] m.p. 58°) was treated with PPE as in the case of XIV The distillate (b.$p_{1.5}$ 165～170°) was recrystallized from hexane to give colorless feather of m.p. 86～87° (lit.,[37] m.p. 88～89°). UV λ_{max}^{EtOH} mμ (log ε): 224 (4.51), 271 (4.21).

3-(3,4-Dimethoxyphenyl)indole (XVII)——3,4-Dimethoxyphenylacetaldehyde was prepared from sodium 3,4-dimethoxyphenylglycidate[38]; b.p_2 125°, semicarbazone, m.p. 158～160°. This aldehyde (10 mmol.) and phenylhydrazine (10 mmol.) were mixed and warmed on a water-bath for few min. to give nearly quantitative amount of the phenylhydrazone of m.p. 97～98° (decomp.) from benzene-hexane. To the solution of the phenylhydrazone (1.15 g.) in $CHCl_3$ (9 ml.) was added PPE (5.5 g.) while shaking. An exothermic reaction took place. After refluxing for 3 min., the solvent was removed *in vacuo*, and cold water (8 ml.) was added under ice-cooling followed by EtOH (12 ml.) to precipitate crude XVII, which was collected, washed, dried and recrystallized from MeOH to form colorless pillars of m.p. 144.5～145.5° (lit.,[39] m.p. 148°). UV λ_{max}^{EtOH} mμ (log ε): 268 (4.29).

3,3-Dimethylindolenine(XIX)——To a solution of isobutyraldehyde phenylhydrazone (b.p_{15} 140～141°; 5,539 mg.) in $CHCl_3$ (20 ml.) was added PPE (25 g.) and the mixture was refluxed for 5 min. After cooling, water (30 ml.) was added and the whole was stirred for 1 hr. under cooling. The organic layer was separated and extracted with 2.5% HCl (20 ml.) and the combined aq. layer was basified with excess of $NaHCO_3$ under cooling, and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated to leave oil, which was solidified by treatment with EtOH; 722 mg. Recrystallization from benzene-EtOH gave colorless fine needles of m.p. 214～215°; 589 mg. or 12% (lit.,[15] m.p. 215～216°; lit.,[16] m.p. 214～215°). Picrate, yellow needles of m.p. 134～135° (lit.,[15] m.p. 135°).

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Summary

Polyphosphate ester (PPE) was demonstrated to be a good agent in the Fischer indole synthesis involving various ketones and aldehydes except ones having methyl group attached to carbonyl, thus providing a convenient preparative route to 2-unsubstituted and 2,3-disubstituted indole and indolenine derivatives.

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36) R. H. Cornforth, Sir R. Robinson: *Ibid.*, **1942**, 680.
37) E. Fischer, T. Schmidt: Ber., **21**, 1811 (1888).
38) Y. Ban, T. Oishi: This Bulletin, **6**, 574 (1958).
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[Chem. Pharm. Bull. 14(9) 939～947 (1966)]

UDC 612.015.3 : 547.562.4.09

127. Hidetoshi Yoshimura, Hiroshi Tsuji, and Hisao Tsukamoto: Metabolism of Drugs. LI.*1 The Metabolic Fate of Alkylaryl Ethers in Rabbits.*2

(*Faculty of Pharmaceutical Sciences, Kyushu University*[*3])

It has been generally accepted that the alkylaryl ethers are cleaved oxidatively into phenols and aldehydes through their hemiacetal intermediates by the enzyme systems which are localized in liver microsomes and require both reduced nicotinamide-adenine dinucleotide phosphate ($NADPH_2$) and oxygen.[1]

*1 Part L: This Bulletin, **12**, 1151 (1964).
*2 A part of this work was shortly communicated in ref. 3).
*3 Katakasu, Fukuoka (吉村英敏, 辻　宏, 塚元久雄).
1) R. T. Williams: "Detoxication Mechanisms," 2 nd ed., p. 324. Chapman & Hall, London (1959).

In the dealkylation study on a series of alkyl *p*-nitrophenyl ethers, McMahon, *et al.*[2] found recently that the ethers of longer alkyl groups, butyl and hexyl, decreased markedly their *in vivo* dealkylation rates in rats in comparison with those of shorter ones, methyl, ethyl, propyl, and isopropyl groups.

This finding suggested that the animal might have alternative pathway rather than dealkylation reaction to metabolize these ethers of longer alkyl groups. In fact, the authors found that (ω-1)-hydroxylation play the most important role in the metabolism of butylaryl ethers, while dealkylation does only a minor role.[3]

The present paper provides the detailed data supporting this conclusion and also deals with the metabolic investigation on other related alkylaryl ethers in rabbits *in vivo*, which throws some light on the relationship between length of alkyl chain of alkylaryl ethers and their metabolic patterns.

Experimental

Materials

Syntheses of 2-Alkoxy-5-nitroanisoles——2-Ethoxy-5-nitroanisole was synthesized by ethylation of 4-nitroguaiacol[4] with diethylsulfate in 10% NaOH on a boiling water bath according to the synthetic method of anisole.[5]

2-Propoxy- and 2-butoxy-5-nitroanisole were prepared by heating 4-nitroguaiacol with corresponding alkyl bromide at 130~140° for 3 hr. in a sealed tube.

Syntheses of 2-Alkoxy-5-nitrophenols——2-Ethoxy,- 2-propoxy,- and 2-butoxy-5-nitrophenol were obtained by hydrolysis of the corresponding 2-alkoxy-5-nitroanisoles with satd. HBr solution in AcOH ac-

Table I. The Melting Points (or Boiling Points), Ultraviolet Absorption Maxima, and Elementary Analyses of Alkylaryl Ethers

Compound	m.p. (b.p.) (°C)	UV λ_{max} mμ (log ε) in EtOH	in 0.1*N* KOH	Analysis Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
2-Ethoxy-5-nitroanisole	85	240 (3.95), 338 (3.88)	—	54.82	5.62	7.10	54.89	5.60	7.48
2-Propoxy-5-nitroanisole	72	240 (4.02), 338 (3.97)	—	56.86	6.20	6.63	56.96	6.12	6.63
2-Butoxy-5-nitroanisole	56	240 (3.98), 338 (3.90)	—	58.60	6.69	6.27	58.65	6.71	6.22
2-Ethoxy-5-nitrophenol	114	244 (3.92), 302 (3.71), 344 (3.81)	227 (3.98), 264 (4.06), 323 (3.67), 418 (3.58)	52.46	4.95	7.65	52.64	4.84	7.66
2-Propoxy-5-nitrophenol	87	244 (3.99), 300 (3.79), 343 (3.86)	227 (3.98), 264 (4.07), 324 (3.76), 420 (3.57)	54.82	5.62	7.10	54.91	5.50	7.40
2-Butoxy-5-nitrophenol	60	244 (4.01), 302 (3.81), 344 (3.91)	227 (4.01), 264 (4.10), 324 (3.76), 418 (3.72)	56.86	6.20	6.63	56.91	6.33	6.69
Propyl *p*-nitrophenyl ether	(163/19 mm.)	228 (3.85), 309 (4.06)	—	—	—	—	—	—	—
Butyl *p*-nitrophenyl ether	32	228 (3.88), 309 (4.06)	—	—	—	—	—	—	—
Isoamyl *p*-nitrophenyl ether	(165/18 mm.)	225 (4.00), 318 (4.08)	—	—	—	—	—	—	—

2) R.E. McMahon, H.W. Culp, J. Mills, F.J. Marshall: J. Med. Chem., **6**, 343 (1963).
3) H. Tsukamoto, H. Yoshimura, H. Tsuji, T. Watabe: This Bulletin, **12**, 987 (1964).
4) T. Watabe, H. Yoshimura, H. Tsukamoto: This Bulletin, **12**, 1151 (1964).
5) "Organic Syntheses," Coll. Vol., **I**, 58.

cording to the method of hydrolysis of 2-ethoxy-5-nitrophenetole.[6]

Syntheses of Alkyl *p*-Nitrophenyl Ethers——Propyl, butyl, and isoamyl *p*-nitrophenyl ethers were synthesized by analogous synthetic method to that of butyl *o*-nitrophenyl ether.[7]

The melting points (or boiling points) and the maxima of ultraviolet absorption spectra of all the compounds described above are summarized in Table I. Some of them were also checked by elementary analyses, the data of which are shown in the same table.

Preparation of β-Glucuronidase——It was obtained from preputial glands of female adult rats (Wistar King) by its homogenation in deionized water. The activity was of $15\sim35\times10^4$ *p*-nitrophenyl-β-D-glucopyranosiduronic acid units[8] per ml. of the homogenate.

Methods

Administration of the Compouds——The alkylaryl ether (900 mg.) was suspended in 10 ml. of 10% gum arabic and given by stomach tube to male albino rabbits weighing about 3.0 kg.

Extraction of Urinary Metabolites——The 48 hr. urine from 3 rabbits, each of which received orally 900 mg. of a desired alkylaryl ether, was boiled in 5% HCl final concentration for one hr. to hydrolyze the conjugated metabolites. Only in the experiment with isoamyl *p*-nitrophenyl ether, the urine was adjusted to pH 4.0 with AcOH and incubated with β-glucuronidase at 38° for 20 hr. instead of acid hydrolysis, since the recovery of hydroxy-metabolite of isoamyl *p*-nitrophenyl ether after acid hydrolysis was considerably low, probably owing to the acid lability of the metabolite.

This hydrolysate with either acid or β-glucuronidase was extracted continuously with ether for 20 hr. The extract was dried over anhyd. Na_2SO_4 and the solvent was distilled off. The residue was dissolved in benzene and this solution was extracted with 5% KOH and washed once with water to obtain a benzene solution containing the neutral metabolites.

The alkaline extract and washing were combined and the mixture was saturated with NaCl, acidified with dil. HCl, and extracted with benzene to yield the acidic fraction.

Each of these two fractions was dried over anhyd. Na_2SO_4 and used for isolation and identification of the metabolites.

Determination of Urinary Metabolites——The ether extract of the hydrolyzed urine from 3 rabbits was dissolved in 50 ml. of EtOH and the solution was submitted to determination procedure of metabolites as follows:

1) ***p*-Nitrophenol**——An aliquot (1 or 2 ml.) from the EtOH solution described above was evaporated to dryness and the solution of this residue in 10 ml. of 0.1 *N* KOH was washed with 3 ml. of $CHCl_3$. *p*-Nitrophenol was determined spectrophotometrically at 400 mμ after dilution of above solution with 0.1 *N* KOH to obtain the appropriate concentration of *p*-nitrophenol. The recovery of this method was about 92%.

2) **(ω-1)-Hydroxy-metabolites**——An aliquot (1~5 μl.) from the EtOH solution of the urine extract mentioned above was submitted directly to gas chromatographic analysis. The condition adopted was described below.

The amount of each hydroxy-metabolite was calculated from its peak area of the gas chromatogram. The relation between the peak area and the amount of (ω-1)-hydroxy-metabolites was proved to be in a good proportion and the recovery was shown to be almost quantitative.

3) **2-Carboxyethyl *p*-Nitrophenyl Ether and 2-(2-Carboxyethyl)-5-nitroanisole**——An aliquot (0.5 ml.) from the EtOH solution of the urine extract was evaporated to dryness and 3.5 ml. of CH_2N_2 solution in ether was added to this residue, allowing to stand for 1 hr. The ether was then evaporated and the residue was dissolved in 5 ml. of EtOH. Two to five μl. of the solution was submitted to gas chromatographic analysis. The recovery was also quantitative.

4) ***p*- and *m*-Dealkylated Metabolites of 2-Alkoxy-5-nitroanisoles**——For determination of phenolic-metabolites of 2-propoxy-and 2-butoxy-5-nitroanisole, 5 ml. of an aliquot from the EtOH solution of the urine extract was evaporated to dryness and dissolved in 10 ml. of benzene. It was then shaken 4 times with 0.1*N* NaOH (15, 10, 10, and 5 ml. each). The combined alkaline extracts were washed with 10 ml. of $CHCl_3$, acidified with dil. HCl, saturated with NaCl, and extracted 3 times with every 40 ml. of $CHCl_3$. The residue remained after evaporation of the solvent from this extract was dissolved in 2 ml. of EtOH and an aliquot (0.1 or 0.2 ml.) was submitted to paper chromatographic separation and each metabolite was determined spectrophotometrically after the extraction of the corresponding spot with 0.1 *N* KOH from the chromatogram in a manner described in the previous paper.[9] The recovery of the metabolites by this procedure was 70 to 75%.

For determination of two phenolic metabolites of 2-ethoxy-5-nitroanisole, 0.1 ml. of an aliquot from the EtOH solution of the urine extract was submitted directly to paper chromatography, followed by spectrophotometric determination, the recovery of which was nearly quantitative. The wave lengths used for spectrophotometry were 435 mμ for 4-nitroguaiacol, and 418 mμ for 2-ethoxy-, 2-propoxy- and 2-butoxy-5-nitro-

6) F. Pollecoff, R. Robinson: J. Chem. Soc., **111**, 932 (1917).
7) "Organic Syntheses", Coll. Vol., **III**, 140 (1955).
8) K. Kato, *et al.*: This Bulletin, **8**, 329 (1960).
9) H. Tsukamoto, H. Yoshimura, T. Watabe: Biochem. Pharmacol., **13**, 1499 (1964).

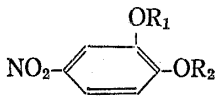
phenol.

Paper and Thin-layer Chromatography——Paper chromatography was carried out in the ascending technique with Toyoroshi No. 51 A filter paper and the solvent system used was butanol, pyridine, benzene, 10% NH_4OH (8:8:5:3).

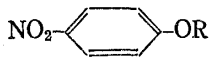
Thin-layer chromatography was carried out by use of silica gel plates, 0.25 mm. thick (Silica gel G, Merck)which was activated at 100° for 60 min. The solvent system used was petroleum ether, benzene, methanol (6:3:1).

The chromatograms were visualized by an ultraviolet lamp (long wave "Manaslu Light", Manaslu Chem. Ind. Co., Ltd., Tokyo). For detection of phenolic metabolites, 5% KOH solution was also adopted. The Rf values are shown in Table II.

Table II. Rf Values of Paper (PPC) and Thin-layer Chromatography (TLC)



R_1	R_2	PPC
CH_3	C_2H_5	0.92
CH_3	H	0.44
H	C_2H_5	0.75
CH_3	n-C_3H_7	0.91
H	n-C_3H_7	0.87
CH_3	$-CH_2\underset{\displaystyle OH}{\underset{\vert}{C}}HCH_3$	0.91
CH_3	$-CH_2CH_2COOH$	0.31
CH_3	n-C_4H_9	0.95
H	n-C_4H_9	0.95
CH_3	$-CH_2CH_2\underset{\displaystyle OH}{\underset{\vert}{C}}HCH_3$	0.95



R	PPC	TLC
n-C_3H_7	0.96	0.90
H	0.48	0.10
$-CH_2\underset{\displaystyle OH}{\underset{\vert}{C}}HCH_3$	0.95	0.30
$-CH_2CH_2COOH$	0.36	—
n-C_4H_9	0.97	—
$-CH_2CH_2\underset{\displaystyle OH}{\underset{\vert}{C}}HCH_3$	0.96	—
iso-C_5H_{11}	0.96	0.90
$-CH_2CH_2\underset{\displaystyle OH}{\underset{\vert}{C}}\begin{matrix}CH_3\\CH_3\end{matrix}$	0.96	0.25

Gas Chromatography——The instrument used for this work was a Shimadzu Model GC-1B equipped with a hydrogen flame ionization detector (dual column and differential flame type). The column was stainless steel U-tube (2.25 m. × 4 mm.). The packing was 1.6% SE-30 on chromosorb W (60~80 mesh), which was pretreated with hexamethyldisilazane. The column temperature was 180°, the injection port temperature 290°, and the detector cell temperature 180°. Nitrogen was used for the carrier gas with a flow rate of 40 ml./min.

Results

Detection, Isolation and Identification of Metabolites

1) **2-Ethoxy-5-nitroanisole**——The ether extract of the hydrolyzed urine described under Methods was investigated by paper chromatography. By spraying 5% KOH solution, two yellow spots were detected on the chromatogram having Rfs of 0.44 and 0.75, which showed the same coloration and mobility with 4-nitroguaiacol and 2-ethoxy-5-nitrophenol, respectively. The same spots were also visualized by ultraviolet lamp, but unchanged 2-ethoxy-5-nitroanisole was not detected.

This extract was then dissolved in benzene and chromatographed on a column packed with 60 g. of silica gel. The yellow band which contained both metabolites was eluted with benzene, and the solvent was evaporated from this fraction to dryness. The residue (1.1 g.) was dissolved in a small amount of MeCH, spotted on 16 sheets of filter paper, 40×40 cm., and developed for 13 to 16 hours in a manner described under Methods.

The areas corresponding to two metabolites on the chromatogram were marked under ultraviolet lamp, cut into pieces, and extracted separately with ether in a Soxhlet apparatus for 20 hours.

The extracted crude metabolite showing Rf 0.44 was recrystallized from benzene to bright yellow needles, m.p. 101~102° (1.0 g.). The ultraviolet and infrared absorption spectra of this metabolite were identical with those of authentic 4-nitroguaiacol and the mixed melting point of both compounds was not depressed.

The other metabolite obtained from the area of Rf 0.75 was also recrystallized from benzene to slightly yellow needles, m.p. 110~111° (100 mg.). The identity of this metabolite with authentic 2-ethoxy-5-nitrophenol was shown by mixed melting point test and by absorption spectra of ultraviolet and infrared.

2) **2-Propoxy-5-nitroanisole**——The urine extract was separated into two fractions, acidic and neutral, by the procedure described under Methods and each fraction was examined separately.

Acidic Fraction——On the paper chromatogram of this fraction, three spots having Rf of 0.30, 0.44, and 0.71 were detected by ultravilolet lamp. The last two spots were colored by spraying with 5% KOH and had the same Rf values with those of 4-nitroguaiacol and 2-propoxy-5-nitrophenol, respectively.

In order to isolate these metabolites, this fraction (0.7 g.) was dissolved in benzene and purified by silica gel column chromatography. Two metabolites having Rfs of 0.44 and 0.71 were first eluted with benzene as a mixture, and another having Rf of 0.30 was then eluted with ether.

The benzene effluent was submitted to paper chromatographic separation, followed by extraction of each metabolite area by the analogous procedure with that of two phenolic metabolites produced from 2-ethoxy-5-nitroanisole. The crude extract (150 mg.) obtained from the area of Rf 0.44 was rechromatographed on silica gel column with benzene as an effluent solvent. Crude crystals obtained from the yellow eluate were recrystallized from benzene to bright yellow needles, m.p. 101~102° (138 mg.) which was identical in every detail with 4-nitroguaiacol.

The other metabolite with Rf 0.71 was similarly isolated as slightly yellow needles, m.p. 86~87° (130 mg.). *Anal.* Calcd for $C_9H_{11}O_4N$: C, 54.82; H, 5.62; N, 7.10. Found : C, 55.15; H, 5.59; N, 7.21. The melting point was not depressed on admixture with authentic 2-propoxy-5-nitrophenol, and the ultraviolet and infrared absorption spectra were also superimposable with those of authentic sample.

The ether effluent which contained only one metabolite described above gave colorless crystals on evaporation of the solvent. It was recrystallized from benzene to colorless scales, m.p. 137~138° (154 mg.). *Anal.* Calcd. for $C_{10}H_{11}O_6N$: C, 49.79; H, 4.60; N, 5.81. Found : C, 49.58; H, 4.55; N, 5.66. UV λ_{max}^{EtOH} mμ (log ε) : 239 (3.99), 336 (3.89); $\lambda_{max}^{0.1N\ KOH}$ mμ (log ε): 229 (3.94), 264 (3.89), 327 (3.76). IR λ_{max}^{KBr} μ : 5.87 ($\nu_{C=O}$), 6.28 ($\nu_{C=C}$), 6.58, 7.45 (ν_{NO_2}).

In addition to the acidic nature, this metabolite possessed a carboxylic band at 5.87 μ in the infrared absorption spectrum. The similar ultraviolet absorption spectrum with that of original compound and the data of elementary analyses also supported that this metabolite should be 2-(2-carboxyethoxy)-5-nitroanisole.

Neutral Fraction——By paper chromatography of this fraction only one spot was detected at Rf 0.72. This Rf value was very similar to that of unchanged 2-propoxy-5-nitroanisole, however, gas chromatographic examination of these two materials excluded the possibility of their identity (see Fig.1).

This neutral fraction (0.75 g.) was then dissolved in benzene and purified by alumina column chromatography. The effluent with a mixture of benzene, ether (9:1) containing above metabolite was collected and the residue obtained after evaporation of

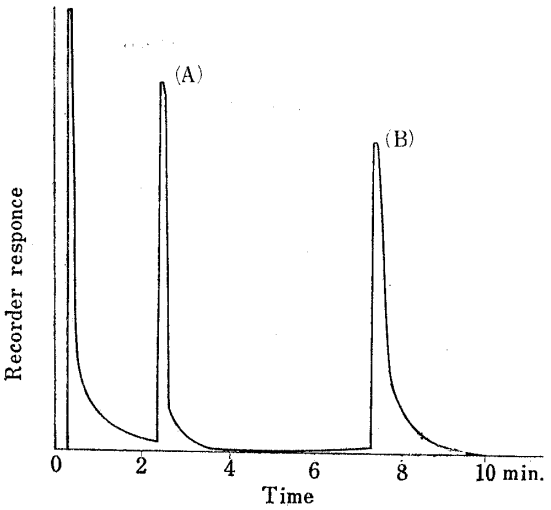


Fig. 1. Gas Chromatogram of 2-Proxy-5-nitroanisole (A) and 2-(2-Hydroxypropoxy)-5-nitroanisole (B)
The condition used was described in the text.

the solvent was recrystallized from MeOH to slightly yellow needles, m.p. 109~110° (274 mg.). $[\alpha]_D^{18}$ +3° (c=3.0, $CHCl_3$). *Anal.* Calcd. for $C_{10}H_{13}O_5N$: C, 52.86; H, 5.77; N, 6.17. Found: C, 52.81; H, 6.01; N, 5.97. UV λ_{max}^{EtOH} mμ (log ε): 238 (4.09), 335 (4.01). IR λ_{max}^{KBr} μ: 2.80 (ν_{OH}), 6.26 ($\nu_{C=C}$), 6.59, 7.46 (ν_{NO_2}). The infrared absorption spectrum clearly indicated the presence of a hydroxyl group. The data of elementary analyses were also agreed with the hydroxylated structure of the metablite, in which one of the carbon atoms of propyl chain would be oxidized to hydroxypropyl group. The position of this hydroxyl group was confirmed by the positive iodoform reaction, which indicated the existence of CH_3CHOH-grouping. The structure of this metabolite, therefore, should be 2-(2-hydroxypropoxy)-5-nitroanisole.

3) **2-Butoxy-5-nitroanisole**

Acidic Fraction——Two distinct spots were observed at Rfs 0.95 and 0.44 and very faint one at Rf 0.75 on the paper chromatogram of this fraction. The first two spots were shown to have the same Rf values with those of authentic 2-butoxy-5-nitrophenol and 4-nitroguaiacol, respectively. The last one was seemed to be very minor component from its faint visualization, and so not investigated further.

This fraction (0.5 g.) was submitted to silica gel column chromatography. When it was eluted with benzene, a metabolite having Rf 0.95 came out first and followed by a mixture of both metabolites (Rfs 0.95 and 0.44). Another metabolite having Rf 0.44 was obtained from the following effluent. The fraction of metabolites mixture was further submitted to paper chromatographic separation followed by extraction of the metabolite areas of paper chromatogram according to the procedure adopted for separation of the phenolic metabolites of 2-propoxy-5-nitroanisole.

Two metabolites of Rfs 0.95 and 0.44 thus obtained were recrystallized from benzene to slightly yellow needles, m.p. 59~60° (68 mg.) and to bright yellow needles, m.p. 101~102° (51 mg.), respectively. They were shown to be identical with 2-butoxy-5-nitrophenol and 4-nitroguaiacol, respectively, by the mixed melting point examination and the comparison of their ultraviolet and infrared absorption spectra.

Neutral Fraction——The paper and gas chromatographic examinations of this fraction indicated the existence of only one metabolite showing Rf 0.95 and RT 12 min. and no unchanged material (Rf 0.95, RT 5.5 min.).

It was purified by alumina column chromatography. The metabolite eluted with benzene was also recrystallized from benzene to slightly yellow needles, m.p. 89~90° (582 mg.); $[\alpha]_D^{16}$ −10° (c=1.0, $CHCl_3$). The structure of this metabolite was proved to be 2-(3-hydroxybutoxy)-5-nitroanisole (Data supporting this structure were already reported in the previous communication).[3]

4) **Propyl *p*-Nitrophenyl Ether**

Acidic Fraction——There were detected two metabolites at Rfs 0.48 and 0.36 on the paper chromatogram of this fraction. They were separated by silica gel column chromatography, in which a metabolite of Rf 0.48 was first eluted and another of Rf 0.36 was then followed with a solvent mixture of benzene-ether.

The former metabolite was recrystallized from benzene to slightly yellow needles, m.p. 113~114° (490 mg.). The mixed melting point examination and the comparison of ultraviolet and infrared absorption spectra of this metabolite with authentic *p*-nitrophenol showed their complete identity.

The latter metabolite of Rf 0.36 was recrystallized also from benzene to colorless scales, m.p. 116~117° (206 mg.). *Anal.* Calcd. for $C_9H_9O_5N$: C, 51.19; H, 4.30; N, 6.63. Found : C, 51.38; H, 4.34; N, 6.67. UV λ_{max}^{EtOH} mμ (log ε) : 228 (3.89), 307 (4.06). IR λ_{max}^{KBr} μ : 5.90 ($\nu_{C=O}$), 6.18, 6.25 ($\nu_{C=C}$), 6.49, 7.37 (ν_{NO_2}).

Considering from these analytical and spectral data it was concluded that the structure of this metabolite should be 2-carboxyethyl *p*-nitrophenyl ether.

Neutral Fraction——This fraction was shown to contain only one metabolite at Rf 0.95 by the paper chromatography and purified by alumina column chromatography.

The metabolite eluted with benzene-ether (1:1) was recrystallized from benzene to slightly yellow needles, m.p. 78~79° (370 mg.); $[\alpha]_D^{18}$ +25° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_9H_{11}O_4N$: C, 54.82; H, 5.62; N, 7.10. Found : C, 55.16; H, 5.78; N, 7.01. UV λ_{max}^{EtOH} mμ (log ε) : 228 (3.95), 307 (4.08). IR λ_{max}^{KBr} μ : 2.96 (ν_{OH}), 6.20, 626 ($\nu_{C=C}$), 6.59, 7.45 (ν_{NO_2}).

From these data together with the positive iodoform reaction, the stucture was shown to be 2-hydroxypropyl *p*-nitrophenyl ether.

5) **Butyl *p*-Nitrophenyl Ether**——A metabolite of Rf 0.48 (88 mg.) and another of Rf 0.96 (393 mg.) were isolated from the acidic and neutral fractions by silica gel and alumina column chromatographies, respectively, using benzene-ether (1:1) as the effluent solvent. The former metabolite, m.p. 113~114°, recrystallized from benzene was proved to be identical with *p*-nitrophenol and the latter, recrystallized from benzene to colorless needles, m.p. 92~93°, $[\alpha]_D^{22}$ +29° (c=1.0, $CHCl_3$), was shown to be 3-hydroxybutyl *p*-nitrophenyl ether (See the previous short communication[3] with regard to the data supporting this structure). The excretion of unchanged material was undoubtedly excluded by gas chromatography.

6) **Isoamyl *p*-Nitrophenyl Ether**——*p*-Nitrophenol, m.p. 113~114° was isolated from the acidic fractoin similarly with above experiment.

The neutral fraction which contained only one metabolite (Rf 0.96) was purified also by alumina column chromatography same as above and oily metabolite was obtained. UV λ_{max}^{EtOH} mμ (log ε) : 228 (3.94), 308 (4.06). IR $\lambda_{max}^{liq.}$ μ : 2.91 (ν_{OH}), 6.28 ($\nu_{C=C}$), 6.65, 7.42 (ν_{NO_2}). The infrared absorption spectrum indicated the existence of a hydroxyl group. In addition, the similar ultraviolet absorption spectrum with that of original ether and the indication of no optical activity suggested that the (ω-1)-hydroxylated structure was highly possible for this metabolite. If it is true, metabolite could be synthesized by chemical means, *i.e.* chromium trioxide oxidation[10] as follows : To a solution of isoamyl *p*-nitrophenyl ether (2 g.) in 10 ml. of AcOH, a solution of chromium trioxide (1.3 g.) in 5 ml. of acetic acid containing a drop of water was added dropwise at 35~40° and continued heating at the same temperature for 4 hours. After evaporation of the solvent the residue was chromatographed on alumina column. Unchanged compound was first eluted with benzene and a small amount of oily oxidation product, which had the same Rf value with that of the metabolite, was then eluted with benzene-ether (8:2). The ultraviolet and infrared absorption spectra of the metabolite and the synthetic sample were quite identical.

Furthermore, both compounds were converted to the identical crystalline *p*-nitrobenzoate, slightly yellow needles, m.p. 129~130° by usual method. *Anal.* Calcd. for $C_{18}H_{18}O_7N_2$: C, 57.75; H, 4.85; N, 7.48. Found : C, 57.97; H, 4.87; N, 7.48. UV λ_{max}^{EtOH} mμ (log ε) : 263 (4.18), 302 (4.13). IR λ_{max}^{KBr} μ : 5.80 ($\nu_{C=O}$), 6.19, 6.26 ($\nu_{C=C}$), 6.51, 7.45 (ν_{NO_2}).

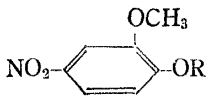
10) E. W. Maynert, J. M. Dawson, E. Washburn : J. Biol. Chem., **195**, 397 (1952).

From above experiments, it was coucluded that the structure of this metabolite should be (3-methyl-3-hydroxy-butyl) *p*-nitrophenyl ether.

Determination of the Urinary Metabolites

Each of the metabolites in the 48 hours urine was determined after hydrolysis with acid or β-glucuronidase according to the procedure described uuder Methods. The results obtained are summarized in Table Ⅲ and Ⅳ

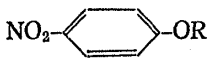
Table Ⅲ. Excretion of the Metabolites in 48 Hours Urine of Rabbits Receiving 2-Alkoxy-5-nitroanisoles



R	Metabolite excreted (% of the doses)			
	p-Dealkylated phenol	*m*-Dealkylated phenol	(ω-1)-Alcohol	ω-Carboxylic acid
CH_3[a)]	54	8	—	—
C_2H_5	41	8	—	—
n-C_3H_7	12	7	18	9
n-C_4H_9	5	5	36	—

a) The data were quoted from the previous paper.[9)]

Table Ⅳ. Excretion of the Metabolites in 48 Hours Urine of Rabbits Receiving Alkyl *p*-Nitrophenyl Ethers



R	Metabolite excreted (% of the doses)		
	p-Nitrophenol	(ω-1)-Alcohol	ω-Carboxylic acid
n-C_3H_7	31	17	16
n-C_4H_9	8	25	—
i-C_5H_{11}	2	39	—

It can be seen from these tables that about half of the administered material was excreted into the 48 hours urine as the identified metabolites.

As reported previously by McMahon, *et al.*, it was also demonstrated that there was a clear correlation between molecular weight and rate of dealkylation : the larger the group, the slower the rate. In this connection, further interesting finding was a rate of (ω-1)-hydroxylation which was inversely related with rate of dealkylation. Rate of the former reaction thus increased in the following order : propyl <butyl <isoamyl.

Propylaryl ethers behaved quite differently with other alkylaryl ethers in respect of producing considerable amount of ω-carboxylic acid.

Discussion

Penultimate hydroxylation is one of the well-established metabolic pathways of many drugs possessing C-alkyl side chain.[11)] However, its occurrence has never been proved in other alkyl compounds involving ethers (O-alkyl) or amines (N-alkyl).

A clear correlation between molecular weight and rate of dealkylation of a series of alkyl *p*-nitrophenyl ethers *in vivo* and *in vitro*, which was elaborated by McMahon, *et al.* prompted us to reinvestigate the metabolism of these alkylaryl ethers, in the

11) B.B. Brodie, J.R. Gillette, B.N. LaDu : Ann. Rev. Biochem., 28, 427 (1958).

hope that the ethers showing slow dealkylation might undergo such penultimate hydroxylation on their O-alkyl groups as on C-alkyl compounds.

By the present investigation it has now been proved that aryl ethers of longer alkyl groups are metabolized mainly through this penultimate hydroxylation pathway and this reaction rate increases in proportion as rate of dealkylation decreases. Methyl or ethyl group in these ethers, thus, underwent quick dealkylation in almost same rate, but longer ones, propyl, butyl or isoamyl group, decreased their dealkylation rates in this order and instead increased the rate of penultimate hydroxylation.

It seemed also very interest that only propoxyl group, intermediate size of alkoxyl, was oxidized on all of the three carbon atoms and produced ω-carboxylic acid together with dealkylation and penultimate hydroxylation products, while in butoxl or isoamyloxy group, no formation of ω-carboxylic acid metabolites was observed.

In the previous study on the metabolism of 4-nitroveratroles,[9] it was found that among the two methoxyl groups, one attached to the para position of nitro-substituent was demethylated preferentially. It was supposed that if this para methoxyl group is replaced by dealkylation-resistant, longer alkoxyl groups and the meta methoxyl is held constant, the major metabolic pathway would be directed to cleavege of the meta methoxyl, but this was shown not to be the case. Rate of meta-demethylation was nearly constant over a series of 2-alkoxy-5-nitroanisoles, and of penultimate hydroxylation of para alkyl group increased as rate of para-dealkylation decreased.

Since the extraction of the urine was carried out all in acidic media, occurrence of reduction of nitro-substituent to amino group could not be ruled out, however it appeared a very minor pathway, if any, considering from the result of the previous study.[9]

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Summary

Major metabolic pathways of two series of alkylaryl ethers, *i.e.* 2-alkoxy-5-nitroanisoles and alkyl *p*-nitrophenyl ethers, were investigated *in vivo* with rabbits.

It was confirmed that aryl ethers of methyl or ethyl were dealkylated quickly, but of butyl or of isoamyl only with slow rate and metabolized mainly through penultimate hydroxylation pathway. Propylaryl ethers, on the other hand, behaved very differently, producing ω-oxidation product together with dealkylation and penultimate hydroxylation products. Demethylation rate of the methoxyl group attached to the meta position of nitro-substituent in 2-alkoxy-5-nitroanisoles was held nearly constant over a series of compounds.

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