give the corresponding ethers (XIX), $C_{18}H_{22}O_3$, m.p. 161~163°, IR ν_{max}^{KBr} cm^{-1} : 1710, NMR (τ) : 8.96 (C_{12}-CH_3), shifted signal (C_1-CH_3) of XIII was disappeared, GC, and XX, $C_{18}H_{22}O_3$, m.p. 128~129°, IR ν_{max}^{KBr} cm^{-1} : 1715, NMR (τ) : 8.74 (C_1-CH_3), shifted signal (C_{12}-CH_3) of XVI was disappeared, GC, in good yield.

On the base of the above mentioned results, possible configuration at C_{10} of both the systems (XIII, XVI and their derivatves) could be discussed.

Since C_{10}-OH of the ester (XVI) having methyl deoxypodocarpate enantiomer skeleton is nearly located to the C_{12}-methyl group and also is combined with the C_{12}-methyl group to give the ether (XX), the configuration at C_{10} of XVI and its derivatves have only one possibility (α C_{10}-OH in half chair B-ring, now regardless of conformation of A-ring). On the other way, C_{10}-OH of the isomeric ester (XIII) having the same skeleton as XVI is, in contrast, nealy located to the C_1-methyl group and is combined with the C_1-methyl group to give the corresponding ether (XIX), so C_{10}-OH configuration of XIII and its derivatives could be considered as β-configuration in half chair B-ring.[*5,*8] In other words, stereochemical relationship of both the systems (XIII nnd XVI) is configurational isomer at C_{10}-OH and isomerisation of the configuration at C_{10} ($\beta \rightarrow \alpha$) must be occurred during the lactonization of XII.[*9]

Anyhow, the selective oxidation of the C_1- and C_{12}-methyl group of these type componds is accomplished. Now, using these compounds, syntheses of some diterpenoids and studies on stereochemical problems of XIII and XVI system are still in progress.

The authors are indebted to Prof. Emeritus E. Ochiai, Tokyo University, for his valuable advice and encouragement.

RIKAGAKU KENKYUSHO
(Institute of Physical and Chemical Research), Kamifujimae-cho, Bunkyo-ku, Tokyo

Akira Tahara (田原 昭)
Ken-ichi Hirao (平尾健一)

Received June 12, 1964

*8 Other possibility on C_{10}-OH configuration (it is α C_{10}-OH in half boat B-ring and thus, stereochemical relationship of both the systems (XIII and XVI) is conformational isomer at B-ring) will be cancelled firmly in our future report.

*9 Authors thank to Dr. W. Nagata, Shiongi & Co., Ltd., Osaka for discussion on stereochemistry of the isomeric compounds.

[Chem. Pharm. Bull. 12 (8) 987 ~ 989]

UDC 612.015.3 : 547.56'58

The Occurrence of (ω-1)-Hydroxylation as the Major Metabolism of Alkylaryl Ethers in Rabbits

In studies on the metabolic fate of a wide variety of alkylaryl ethers by a number of workers, it has been elaborated that the ethers can be cleaved oxidatively to phenols and aldehydes by the enzyme systems locating in liver microsomes and requiring both reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and oxygen.[1] This O-dealkylation has been known as a sole metabolic pathway of alkylaryl ethers (Chart 1).

$$Ar\text{-}O\text{-}CH_2\text{-}R \xrightarrow[NADPH,\ O_2]{\text{liver microsomes}} \left[\begin{array}{c} OH \\ | \\ Ar\text{-}O\text{-}CH\text{-}R \end{array}\right] \longrightarrow Ar\text{-}OH + RCHO$$

Chart 1.

1) R. T. Williams : "Detoxication Mechanisms," 2nd ed., p. 324. Chapman & Hall, London (1959).

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

From this fact, the authors presumed that the animal might have alternative pathways to metabolize these ethers of longer alkyl groups, because they have a high lipid-solubility and therefore would be retained for a long time in the body and do harm to the animal unless they would be converted into more polar, water-soluble cmpounds.

The present communication will provide the first evidence that butylaryl ethers undergo only a small amount of ether cleavage and instead, mainly (ω-1)-hydroxylation of butyl group in rabbits.

The 48 hour-urine from three male rabbits weighing about 3.0 kg., each of which received orally 900 mg. of butyl *p*-nitrophenyl ether (suspended in gum arabic), was boiled in 5% hydrochloric acid concentration for 30 minutes to hydrolyze the conjugated metabolites and extracted continuously with ether for 24 hours. The extract was separated into two fractions, neutral and acidic by usual method.

The neutral fraction, after purification by alumina column chromatography, gave colorless needles, m.p. 93° (393 mg.; recrystallized from benzene); $[\alpha]_D^{22}$ +29° (c=1.0 in $CHCl_3$). *Anal.* Calcd. for $C_{10}H_{13}O_4N$: C, 56.94; H, 6.08; N, 6.88. Found: C, 56.91; H, 6.20; N, 6.63. UV λ_{max}^{EtOH} mμ (ε): 228 (8,400), 309 (13,500). IR $\lambda_{max}^{CHCl_3}$ μ: 2.75 (ν_{O-H}); 6.20, 6.26 ($\nu_{C=C}$); 6.66, 7.40 (ν_{NO_2}); 11.85 (δ_{C-H}).

The elementary analysis showed a good agreement with hydroxybutyl *p*-nitrophenyl ether. The neutral property of the metabolite ruled out a phenolic structure, and the observation of optical activity strongly suggested that a hydroxyl group should be located on one of methylenes of butyl group. The ultraviolet and infrared absorption spectra also indicated that there should not be any change in aromatic moiety, but a newly formed hydroxyl group on alkoxyl group. The positive iodoform reaction for this metabolite indicated the existence of CH_3CHOH-grouping. Therefore it was confirmed that the structure of this major metabolite should be 3-hydroxybutyl *p*-nitrophenyl ether.

The acidic fraction was purified through silica gel column chromatography, and a crystalline metabolite (88 mg.) was obtained from the effluents with benzene and benzene-ether (1:1). It was recrystallized from benzene to slightly yellow needles, m.p. 113~114°. UV λ_{max}^{EtOH} mμ (ε): 229 (7,000), 313 (11,100), $\lambda_{max}^{0.1N\ KOH}$: 402 mμ ($\varepsilon$ 17,500). IR λ_{max}^{KBr} μ: 2.96 (ν_{O-H}); 6.19, 6.26 ($\nu_{C=C}$); 6.67, 7.45 (ν_{NO_2}); 11.81 (δ_{C-H}). The melting point was not depressed on admixture with authentic *p*-nitrophenol, and the ultraviolet and infrared absorption spectra were also superimposable with those of authentic sample.

The next attention was directed toward metabolic study on 2-butoxy-5-nitroanisole as the second choice for learning the possibility of occurrence of (ω-1)-hydroxylation of alkylaryl ethers. In our series of *in vivo*[3] and *in vitro*[4] studies on selective demethylation of aromatic compounds possessing two adjacent methoxyl groups, it was found that the methoxyl group attached on the *para* position of nitro substituent of 4-nitroveratrole was cleaved predominantly in rabbits, and therefore it seemed also to be of interest to know how the metabolism would change when the methoxyl group of the *para* position was replaced by longer alkoxyl groups.

The experiment was performed similarly to that of butyl *p*-nitrophenyl ether. The 48 hour-urine from three rabbits administered 900 mg./body of 2-butoxy-5-nitroanisole

2) R. E. McMahon, H. W. Culp, J. Mills, F. J. Marshall: J. Med. Chem., **6**, 343 (1963).
3) H. Tsukamoto, H. Yoshimura T. Watabe: Biochem. Pharmacol., in press.
4) T. Watabe, H. Yoshimura, H. Tsukamoto: This Bulletin, in press.

was submitted to the isolation of metabolites. The major metabolite (582 mg.) was also obtained from the neutral fraction, and recrystallized from benzene to slightly yellow needles, m.p. 89~90°; $[\alpha]_D^{16}$ −10° (c=1.0 in $CHCl_3$). *Anal.* Calcd. for $C_{11}H_{15}O_5N$: C, 54.54; H, 6.66; N, 5.78. Found : C, 54.69; H, 6.34; N, 5.86. UV λ_{max}^{EtOH} mμ (ε) : 241 (9,500), 338 (8,000). IR $\lambda_{max}^{CHCl_3}$ μ : 2.81 (ν_{O-H}); 6.27 ($\nu_{C=C}$); 6.63, 7.40 (ν_{NO_2}); 11.51 (δ_{C-H}). Considering these analytical, optical, and spectral data together with a positive iodoform reaction, it was again concluded that the structure of this metabolite is 2-(3-hydroxybutoxy)-5-nitroanisole.

As the minor metabolites, 2-butoxy-5-nitrophenol, m.p. 59~60° (68 mg.) and 4-nitroguaiacol, m.p. 101~102° (51 mg.) were isolated from the acidic fraction through silica gel column chromatography, followed by paper chromatography*1 on a preparative scale.

The identity of the metabolites with authentic samples was proved by the mixed melting point examination, and the comparison of their Rf values of paper chromatography, and ultraviolet and infrared absorption spectra.

2-Butoxy-5-nitrophenol : UV $\lambda_{max}^{0.1N\ KOH}$ mμ (ε) : 264 (12,500), 324 (5,800), 418 (4,300). IR λ_{max}^{KBr} μ : 2.82 (ν_{O-H}); 6.16, 6.21 ($\nu_{C=C}$); 6.60, 7.42 (ν_{NO_2}).

4-Nitroguaiacol : UV $\lambda_{max}^{0.1N\ KOH}$ mμ (ε) : 265 (5,600), 433 (16,500). IR λ_{max}^{KBr} μ : 2.95 (ν_{O-H}); 6.29 ($\nu_{C=C}$); 6.60, 7.42 (ν_{NO_2}); 11.46 (δ_{C-H}).

Although the presence of a few more metabolites for both butylaryl ethers was also shown by paper chromatography, they were very minor products and excluded from the present discussion.

By these two experiments, it is now evident that (ω-1)-hydroxylation participates the most important role in the metabolism of alkylaryl ethers, while ether cleavage does only a minor role, if alkyl group is butyl or probably longer ones.

Extensive studies are carrying out on the metabolism of other alkylaryl ethers.

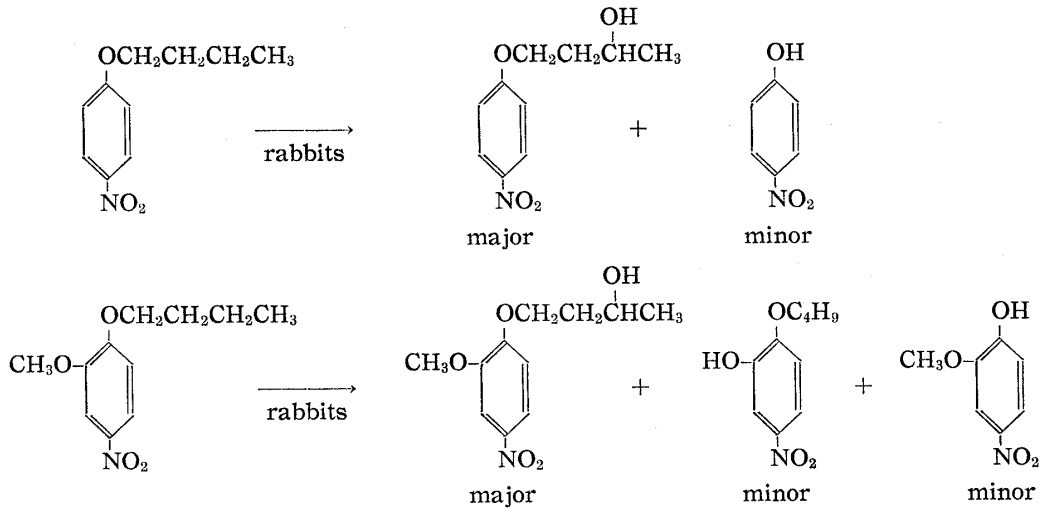

Chart 2.

Faculty of Pharmaceutical Sciences,
Kyushu University,
Katakasu, Fukuoka

Hisao Tsukamoto (塚元久雄)
Hidetoshi Yoshimura (吉村英敏)
Hiroshi Tsuji (辻 宏)
Tadashi Watabe (渡部 烈)

Received June 15, 1964

*1 The solvent system of BuOH-pyridine-benzene-10% NH_4OH (40:40:25:15) and the filter paper, Toyoroshi No. 51A were used. Rf values were 0.95 (2-butoxy-5-nitrophenol) and 0.44 (4-nitroguaiacol), respectively.