Metabolism of 2-Methyl-2-(4-acetaminophenoxy)propane

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The metabolism of the *tert*-butyl analog of acetophenetidin (phenacetin USP XVII) has been investigated. A metabolite which is hydroxylated in the alkyl side chain was isolated.

THE MAJOR metabolic pathway of acetophenetidin (phenacetin USP XVII) involves *O*-dealkylation to *N*-acetyl-*p*-aminophenol (acetaminophen, NF XII) (1). Studies in these laboratories indicated that the *tert*-butyl homolog of acetophenetidin undergoes very little *O*-dealkylation to *N*-acetyl-*p*-aminophenol but that it possesses analgesic and antipyretic activity (2, 3). Although toxicity studies have ruled out the use of this compound as a substitute for acetophenetidin, the authors have investigated its metabolism because these studies could provide interesting data as to structure–metabolism relationships. The identification of a metabolite which is hydroxylated in the side chain is reported.

EXPERIMENTAL

Chemicals—2 - Methyl - 2 - (4 - acetaminophenoxy)propane [*p*-*tert*-butoxy acetanilid] (I) and 2-methyl-2-(4-acetaminophenoxy) propanol (II) were synthesized in these laboratories.[1] *N*-Acetyl-*p*-aminophenol was supplied by Eastman's Distillation Products, Inc., Rochester, N. Y.

Animal Experiments—2-Methyl-2-(4-acetaminophenoxy)propane was administered orally to three adult dogs (50 mg./Kg./day) for 2 days, a total of 3.29 Gm. The urine collected (1270 ml.) during the 2 days was combined. Urine was also collected for the day prior to drug administration.

Chemomatographic Analysis of the Urine—Urine was hydrolyzed and extracted according to the method described earlier in connection with urine obtained from animals treated with acetophenetidin (4). For the preliminary studies, the residue obtained after evaporation of the organic solvent was chromatographed on paper with system *1* previously employed for the separation of acetophenetidin metabolites (4). More recent studies were carried out with thin-layer chromatography as previously described (4). The solvent systems used for thin-layer chromatography are shown in Table I. Visualization of compounds was achieved with ultraviolet light.

Isolation of the Metabolite—A mixture of 800 ml. of urine, 800 ml. of pH 5, 0.2 *N* acetate buffer (5), and 800 ml. of β-glucuronidase[2] was incubated for 18 hr. at 37.5°. The urine was then mixed with 60 ml. of 4 *N* NaOH and 800 ml. of 1.0 *M* phosphate buffer (pH 7.4),[3] and extracted 3 times with 3 L. of $CHCl_3$. The organic extracts were combined and evaporated at room temperature under vacuum. The residue (1.3 Gm.) was dissolved in 5 ml. $CHCl_3$ and chromatographed on an aluminum oxide (10 Gm.) column (acid alumina, DG 4 grade, 100–200 mesh as supplied by Bio Rad Co., Los Angeles, Calif.). The column was washed with 1 ml. of chloroform, 15 ml. of benzene, 150 ml. of benzene–chloroform (35:65 v/v), and 400 ml. of chloroform–ethyl acetate (85:15 v/v). Each fraction was examined chromatographically for the presence of metabolite. The solvent was evaporated from the chloroform–ethyl acetate fraction and the residue (200 mg.) was treated 6 times with 100 ml. of boiling cyclohexane. An insoluble black residue was discarded and the cyclohexane extracts were combined. The cyclohexane was evaporated and the residue was recrystallized 6 times from benzene. More metabolite was obtained from the mother liquors, and a total of 90 mg. of pure product was obtained.

TABLE I—ANALYSIS OF TEST COMPOUNDS BY THIN-LAYER CHROMATOGRAPHY

Compd.	System *1*[a]	System *2*[b]	System *3*[c]
2-Methyl-2-(4-acetaminophenoxy)propane	0.87	0.78	0.94
2-Methyl-2-(4-acetaminophenoxy) propanol	0.23	0.29	0.52
N-Acetyl-*p*-aminophenol	0.08	0.10	0.35

[a] System *1*, previously described (4). [b] The upper phase of a toluene–benzene–water–acetic acid (2:2:1:2) mixture for thin-layer chromatography. [c] The lower phase of a chloroform–methanol–water–acetic acid (20:10:20:1) mixture for thin-layer chromatography.

Acidic Cleavage of Compounds—The acidic cleavage of compounds I and II was done by treating 200 mcg. of each substance with 1 ml. of 2 *N* HCl at room temperature. After a time lapse of 10 min. to 24 hr., portions of the solution were extracted with 10 ml. of a methylene chloride–isopropanol–water (75:25:2) mixture. The solvent was evaporated and the resulting residues were chromatographed on thin-layer plates.

Gas Chromatography—For gas chromatographic analysis of 2-methyl-2-(4-acetylaminophenoxy)propane and the hydroxylated metabolite, urine was enzyme hydrolyzed and extracted as described above. For the analyses of *N*-acetyl-*p*-aminophenol, hydrolyzed urine was extracted with the methylene chloride–isopropanol mixture described above. The analyses were carried out on a Barber-Coleman model 10 gas chromatograph equipped with a Sr-90 detector. The column packing [SE-30, 6% coated on Gas Chrom S, 80–100 mesh supplied by Applied Science Labs., Inc., College Point, Pa., or a mixture of SE-30 (1%)–Carbowax 20 M (1%) coated on Anakrom AS, 80–90 mesh, supplied by Analabs, Inc. Hamden, Conn.] was packed in a U-shaped, glass column, 120 cm. in length and 3.5 mm. i.d.

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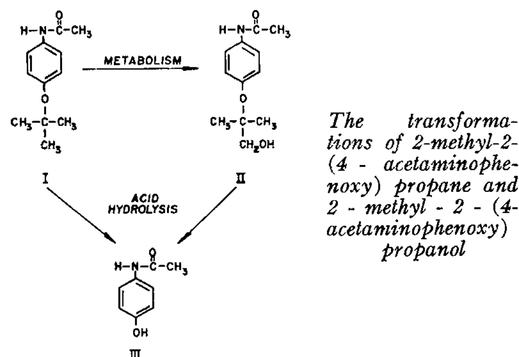
[1] Harfenst, M., to be published.

[2] Ketodase, Warner-Chilcott, Morris Plains, N. J.

[3] KH_2PO_4 (272 Gm.) and 5 *N* KOH (320 ml.) diluted to 2 L. with water.



The transformations of 2-methyl-2-(4-acetaminophenoxy) propane and 2-methyl-2-(4-acetaminophenoxy) propanol

Scheme I

TABLE II—ANALYSIS OF URINE BY GAS CHROMATOGRAPHY

Compd.	Retention Time, min.	Urinary Concn., mcg./ml.	Dose % Recovery in Dogs
2-Methyl-2-(4-acetaminophenoxy)propane	2.1	23	0.9
2-Methyl-2-(4-acetaminophenoxy)propanol[a]	6.1	295	11.4
N-Acetyl-*p*-aminophenol[b]	12.5[c]	101	3.9

[a] The SE-30 column. [b] The SE-30 Carbowax 20 M column. [c] *N*-Acetyl-*p*-aminophenol (retention time = 3 min.) has too broad a peak for accurate quantitation using column a.

The inlet pressure was 25 psig and the outlet pressure was atmospheric. The instrument was operated isothermally at 150° for the SE-30 column and at 200° for the SE-30–Carbowax column. The samples, dissolved in 2–5 μl. of ethyl acetate varied from 0.5–1.5 mcg. The concentration of compound was determined from the proportion of peak area of unknown to peak area of standard (appropriate reference compound added to control urine). Peak areas were calculated from the product of peak height and width at one-half the peak height.

RESULTS AND DISCUSSION

Initial chromatographic studies on extracts of urine obtained from dogs which had received oral doses of 2-methyl-2-(4-acetaminophenoxy)propane, indicated the presence of a metabolite. The chromatographic mobility of the substance was different from that of drug or *N*-acetyl-*p*-aminophenol. Since the compound was freed by treatment with β-glucuronidase it appeared likely that it was hydroxylated.

In order to identify the metabolite, it was isolated as described under *Experimental*. The crystals obtained melted at 120.5–121.5°. The elemental analysis for carbon-hydrogen was in agreement with a hydroxylated derivative of the *tert*-butoxy compound administered.

Anal.—Calcd. for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.68. Found: C, 64.90; H, 7.70.

The location of the hydroxyl group was now considered. Since the metabolite did not react with the Pauly reagent (6), it appeared that the compound was hydroxylated in the side chain.

The metabolite and the drug administered were subjected to mild acid hydrolysis and the products were examined chromatographically. The drug was hydrolyzed completely to *N*-acetyl-*p*-aminophenol in about 15 min. and the metabolite gave about an 80% yield of the same phenol after 24 hr. Since the same product was formed from both, it was established that the hydroxylation was associated with the alkyl moiety.

Authentic 2-methyl-2-(4-acetaminophenoxy) propanol (II) was now synthesized[1] and the metabolite and the authentic synthetic sample were shown to be identical in the following way. A mixed melting point determination carried out on the metabolite and the synthetic compound showed no depression of the melting point. The infrared spectra of the metabolite and of II were identical.

The chromatographic mobilities of the three compounds are shown in Table I.

The structures of 2-methyl-2-(4-acetaminophenoxy)propane (I), the metabolite (II), and *N*-acetyl-*p*-aminophenol (III) are shown in Scheme I.

During the studies on the metabolism of 2-methyl-2-(4-acetaminophenoxy) propane, small amounts of a substance with the chromatographic mobility of *N*-acetyl-*p*-aminophenol were observed. Gas chromatography was utilized to measure the amounts of unchanged drug, the hydroxylated metabolite, and *N*-acetyl-*p*-aminophenol excreted in the urine. The results are shown in Table II.

The results for the small amount of *N*-acetyl-*p*-aminophenol in the urine of animals treated with the *tert*-butoxy compound contrasts with larger amounts of the dealkylated compound excreted by animals treated with acetophenetidin (1). These results, however, were not investigated further.

The substitution of a *tert*-butyl group in the acetophenetidin molecule inhibited the *O*-dealkylation of this compound. These observations can be applied in the synthesis of potential substitutes for drugs when the slowing down of the metabolic process would help in providing a longer duration of therapeutic action. This approach to the development of new drugs has already been discussed in connection with another group of compounds (7).

REFERENCES

(1) Brodie, B. B., and Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **97**, 58(1949).
(2) Conney, A. H., Sansur, M., Soroko, F., Koster, R., and Burns, J. J., *ibid.*, **151**, 133(1966).
(3) Conney, A. H., Sansur, M., and Harfenist, M., *Pharmacologist*, **7**, 160(1965).
(4) Klutch, A., Harfenist, M., and Conney, A. H., *J. Med. Chem.*, **9**, 63(1966).
(5) "Biochemists Handbook," Van Nostrand and Co., Inc., Princeton, N. J., 1961, p. 31.
(6) "Chromatographic and Electrophoretic Techniques," Smith, I., ed., 2nd ed., vol. I, Interscience Publishers, Inc., New York, N. Y., 1960, pp. 296–297.
(7) Klutch, A., and Bordun, M., *J. Med. Chem.*, to be published.