Metabolites and Analogs of 2-Ethyl-2,3-dihydro-5-benzofuranacetic Acid (Furofenac): Chemical and Pharmacological Properties

CLAUDIO CASALINI *, GIUSEPPE MASCELLANI *x, GIANFRANCO TAMAGNONE *, GIULIA CESARANO *, and ANGELO GIUMANINI ‡

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Abstract □ The *in vivo* metabolism of 2-ethyl-2,3-dihydro-5-benzofuranacetic acid (furofenac), a new drug, was studied in rats, dogs, and humans. The drug has antiplatelet-aggregation activity and anti-inflammatory activity combined with low ulcerogenic power. Hydroxylated derivatives and analogous compounds were prepared, and their syntheses and chemical characteristics are described. TLC, GLC, high-pressure liquid chromatography, and GLC–mass spectrometry were applied to urine extracts, and authentic synthetic specimens were used for comparison. The products identified in human and dog urine were mainly conjugation compounds of the drug, while the products in rat urine were hydroxylated derivatives. Some pharmacological characteristics of the metabolites are discussed.

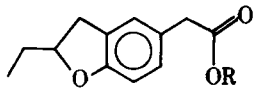
Keyphrases □ 2-Ethyl-2,3-dihydro-5-benzofuranacetic acid—*in vivo* metabolism, rats, dogs, humans □ Drug metabolism—2-ethyl-2,3-dihydro-5-benzofuranacetic acid, *in vivo*, rats, dogs, humans □ Anti-inflammatory agents—2-ethyl-2,3-dihydro-5-benzofuranacetic acid, *in vivo* metabolism, rats, dogs, humans

2-Ethyl-2,3-dihydro-5-benzofuranacetic acid[1] (furofenac, I) was synthesized during a research project aimed at finding compounds with potential anti-inflammatory activity (1, 2). Pharmacotoxicological investigations showed that I has marked anti-inflammatory and antiplatelet-aggregation activity, low toxicity, and very slight ulcerogenicity. It interferes with the biosynthesis of prostaglandins (3), and some *in vivo* pharmacological tests showed that it has good inhibitory activity against thromboxane synthetase (4).

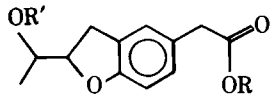
The synthesis of I was optimized (5, 6) in view of clinical trials, and pharmacokinetic studies were undertaken to define its metabolic fate and to search for metabolites of pharmacological interest. The purposes of the present study were to identify and synthesize some of the metabolites of I and to evaluate their anti-inflammatory activity.

EXPERIMENTAL[2]

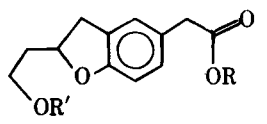
2-(1-Acetoxyethyl)-2,3-dihydrobenzofuran-5-ylacetic Acid Methyl Ester (IV)—2-(1-Acetoxyethyl)-2,3-dihydrobenzofuran (3.3 g, 16 mmoles) (7) was added at 5° to a solution of zinc chloride (2.5 g, 18 mmoles) in acetic anhydride (5 ml) with stirring. The reaction mixture was poured into 0.5 *N* HCl after 4 hr at 5°, and the methylene chloride extract was dried over sodium sulfate and filtered over 70–230-mesh silica gel (5 g). The solvent was distilled off.



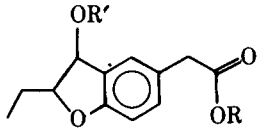
I: R = H
II: R = CH_3



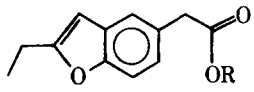
IV: R = CH_3, R′ = CH_3CO
V: R = R′ = H



IX: R = CH_3, R′ = CH_3CO
X: R = R′ = H



XV: R = R′ = H
XVII: R = CH_3, R′ = CH_3CO



XVI: R = H
XVIII: R = CH_3

2-(1-Acetoxyethyl)-5-acetyl-2,3-dihydrobenzofuran (III), bp 155–158°/2 mm Hg, was obtained in a 58% yield. Compound III (1.6 g, 6.5 mmoles) was added at 5° to a solution of thallium trinitrate (3.1 g, 7 mmoles) in methanol (20 ml) and 70% perchloric acid (4 ml). After 3 hr at 5°, the mixture was kept at room temperature for 2 hr. The mixture then was filtered, diluted, and finally extracted with methylene chloride. The solvent was evaporated to give IV (79% yield).

2-(1-Hydroxyethyl)-2,3-dihydrobenzofuran-5-ylacetic Acid (V)—A mixture of IV (1.6 g, 7 mmoles) and sodium bicarbonate (1.6 g, 19 mmoles) in methanol–water was refluxed for 4 hr. It then was cooled to room temperature and extracted with ethyl acetate. The organic layer was washed (2 *N* HCl and water) and dried over sodium sulfate, and the solvent was evaporated.

Crystallization of the residue from hexane–methylene chloride gave pure V (78% yield), mp 85–87°; IR: 3400, 2960, 2900, 1690, 1490, 1410, 1255, 1210, and 1085 cm^{-1}; UV (CH_3OH): 232 (log ϵ 3.801) and 286 (3.468) nm; mass spectrum: *m/e* 131 (100%) and 222 (M^+, 13); ^1H-NMR: δ 6.60–7.10 (m, 3), 4.59 (m, 1), 3.85 (m, 1), 3.50 (s, 2), 3.14 (d, 2), and 1.22 (d, 3) ppm.

Anal.—Calc. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 65.06; H, 6.31.

2-(2-Acetoxyethyl)-2,3-dihydrobenzofuran-5-ylacetic Acid Methyl Ester (IX)—A solution of 2,3-dihydrobenzofuran-2-ylacetic acid ethyl ester (6.9 g, 33.5 mmoles) (8) in ether was added to a slurry of lithium aluminum hydride (2.5 g, 66 mmoles) in ether (50 ml) over 30 min. The reaction mixture was refluxed for 10 hr and then chilled and quenched with 2 *N* HCl. 2-(2-Hydroxyethyl)-2,3-dihydrobenzofuran (VI), obtained from evaporation of the ether extract, was treated with acetic anhydride (10 ml) and pyridine (8 ml). The mixture was heated at 40° for 1 hr, and then ether and 0.5 *N* HCl were added. Usual workup gave 2-(2-acetoxyethyl)-2,3-dihydrobenzofuran (VII) (87% yield), bp 140–142°/2 mm Hg. The IR and UV spectra were as expected.

Compound VII (6 g, 29 mmoles) was added to a solution of anhydrous zinc chloride (4.8 g, 36 mmoles) in acetic anhydride (12 ml, 128 mmoles) with stirring over 30 min at 5°. After 2.5 hr at this temperature, the reaction mixture was extracted with ether. The workup gave 2-(2-acetoxyethyl)-5-acetyl-2,3-dihydrobenzofuran (VIII), bp 202–204°/2 mm Hg, mp 43–44°. The IR and UV spectra agreed with the expected structure.

[1] Furofenac has been patented by Schiapparelli, Turin, Italy.

[2] Melting points were determined in open capillary tubes and are uncorrected. IR spectra were obtained with a Perkin-Elmer 257 spectrophotometer in a potassium bromide disk. UV spectra were obtained with a Perkin-Elmer 550 spectrophotometer in methanol. ^1H-NMR spectra were obtained in deuteromethanol with a Jeol 60 HL spectrometer with tetramethylsilane as the internal standard. Mass spectra were obtained with an LKB 9000 gas–liquid chromatograph–mass spectrometer. GLC analyses were carried out on a Perkin-Elmer 3920 instrument equipped with a flame-ionization detector. HPLC analyses were performed on a Perkin-Elmer 601 instrument equipped with a UV/visible variable-wavelength detector. Elemental analyses were performed by Istituto di Chimica degli Intermedi, University of Bologna. All reagents and solvents were of analytical reagent grade and were used without further purification.

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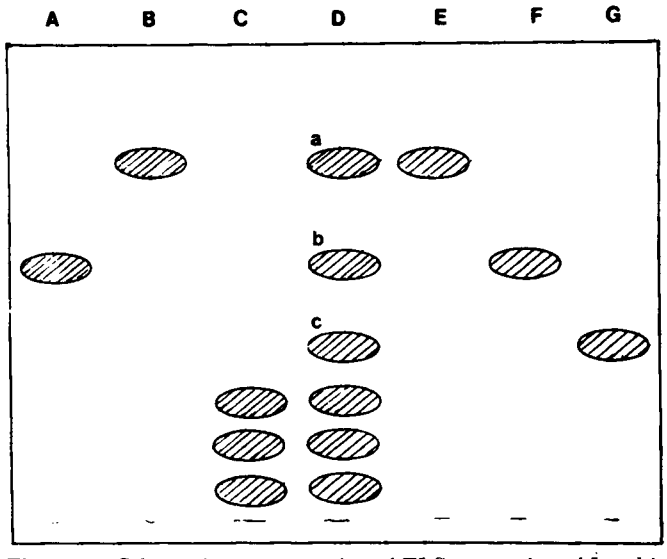


Figure 1—*Schematic representation of TLC separation of I and its metabolites. Key: A, XV; B, I; C, urine blank; D, chloroform extract A of rat urine samples; E, XVI; F, V; and G, X.*

Compound IX was obtained (85% yield) from VIII by treatment with thallium trinitrate as described for the preparation of IV.

2-(2-Hydroxyethyl)-2,3-dihydrobenzofuran-5-ylacetic Acid (X)—Crude IX was hydrolyzed with sodium hydroxide in ethanol–water to afford X (77% yield), mp 111–113° (dichloroethane); IR: 3400, 2920, 1690, 1495, 1410, 1255, 1210, and 1055 cm^{-1}; UV (CH_3OH): 232 (log ϵ 3.823) and 286 (3.475) nm; mass spectrum: *m/e* 222 (M^+, 100%); ^1H-NMR: δ 6.54–7.05 (m, 3), 4.93 (m, 1), 3.73 (t, 2), 3.48 (s, 2), 3.00 (m, 2), and 1.90 (m, 2) ppm.

Anal.—Calc. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.58; H, 6.34.

2-Ethyl-3-hydroxy-2,3-dihydrobenzofuran-5-ylacetic Acid (XV)—Butyryl chloride (10.5 g, 0.1 mole) was added to a solution of *p*-hydroxyphenylacetic acid ethyl ester (18 g, 0.1 mole) and pyridine (7.9 g, 0.1 mole) in methylene chloride (50 ml) over 30 min. The mixture was refluxed for 2 hr and worked up to give *p*-butyryloxyphenylacetic acid ethyl ester (XI).

Compound XI (25 g, 0.1 mole) and aluminum chloride (32 g, 0.24 mole) were heated at 120–130°. The mixture was stirred with 2 *N* HCl, and the precipitate obtained was filtered. Crystallization from carbon tetrachloride yielded 3-butyryl-4-hydroxyphenylacetic acid (XII) (69% yield), mp 115–117°.

Bromine (8.3 g, 52 mmoles) was added to a solution of XII (11.1 g, 0.05 mole) in dichloroethane (60 ml) over 2.5 hr. After standing overnight, the solution was treated with hexane to afford 3-[(α-bromo)butyryl]-4-hydroxyphenylacetic acid (XIII) (88% yield), mp 135–137°.

Treatment of XIII (13 g, 43 mmoles) with a solution of sodium bicarbonate (13 g, 0.15 mole) in water (90 ml) gave 2-ethyl-3-oxo-2,3-dihydrobenzofuran-5-ylacetic acid (XIV) (78%), mp 118–120° (cyclohexane–ethyl acetate).

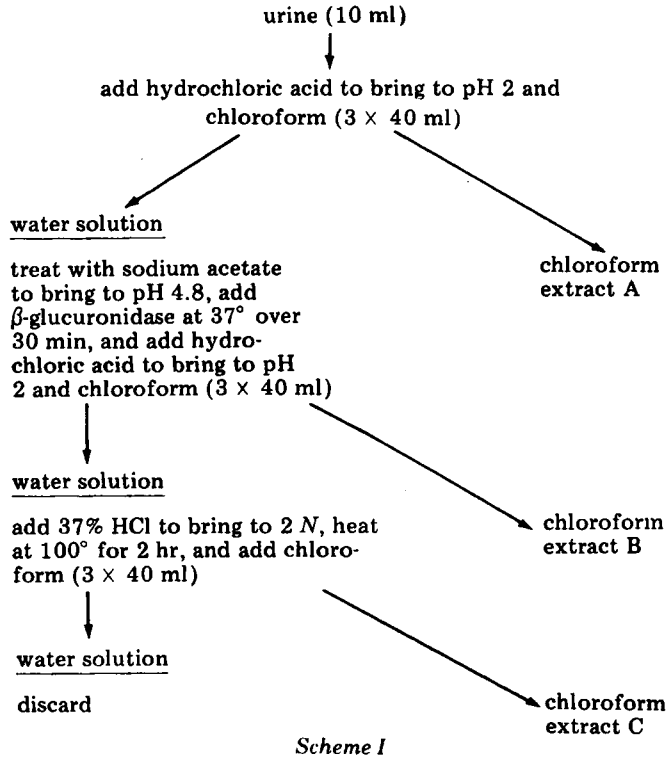
A solution of XIV (6.6 g, 33 mmoles) and sodium bicarbonate (2.8 g, 33 mmoles) in 30 ml of water was treated slowly with sodium borohydride (0.8 g, 21 mmoles). After 1.5 hr at room temperature, the reaction mixture was quenched with ethanol (8 ml) and chloroacetic acid (8.5 g, 90 mmoles) to give XV, mp 122–123° (80% yield); IR: 3200, 2940, 1680, 1625, 1485, 1415, 1300, 1270, 1240, 1210, 1145, 1060, 1025, 980, 925, 890, 815, 785, 755, and 710 cm^{-1}; UV: 232 (log ϵ 3.850) and 286 (3.426) nm; ^1H-NMR: δ 7.30–6.60 (m, 3), 4.20 (m, 1), 3.42 (s, 2), 1.70 (m, 2), and 1.00 (t, 3) ppm; mass spectrum: *m/e* 189 (100%) and 222 (M^+, 98).

Anal.—Calc. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 65.25; H, 6.26.

After heating in 2 *N* HCl for 0.5 hr, XV was dehydrated to XVI.

2-Ethylbenzofuran-5-ylacetic Acid (XVI)—*N*-Bromosuccinimide (1.8 g, 10 mmoles) was added over 30 min to a carbon tetrachloride solution (30 ml) of I (2.05 g, 10 mmoles) and a catalytic amount of benzoyl peroxide. The mixture was refluxed for 1 hr, cooled, and filtered on a small silica gel column, from which XVI was eluted with methylene chloride–methanol (50:1, v/v). Pure XVI was obtained (60% yield) by crystallization from cyclohexane, mp 90–92°; IR: 2960, 2900, 1690, 1590, 1465, 1410, 1295, 1265, 1235, 1190, 1150, and 930 cm^{-1}; UV: 249 (log ϵ 4.129), 281 (3.514), and 288 (3.537) nm; mass spectrum: *m/e* 159 (100%) and 204 (M^+, 71); ^1H-NMR (*d*-chloroform): δ 10.86 (s, 1), 7.10–7.48 (m, 3), 6.38 (s, 1), 3.70 (s, 2), 2.81 (s, 2), and 1.30 (t, 3) ppm.

Anal.—Calc. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.90; H, 5.99.



Scheme I

***In Vivo* Metabolism**—Adult male rats (Wistar), 250 g, and male beagle dogs, ~12 kg, were fed normally during drug administration. The cages were equipped with a urine–feces separator. No metabolic inducers were used. Tests also were performed on human volunteers in good health.

Compound I was administered orally in single doses to rats (500 mg/kg in arabic gum), dogs (100 mg/kg in a jelly capsule), and human volunteers (6.5 mg/kg in a hard jelly capsule). Individual 24-hr urine was collected after administration.

Urine Extraction—The drug and metabolites were extracted from collected urine according to Scheme I. Each chloroform extract was dried

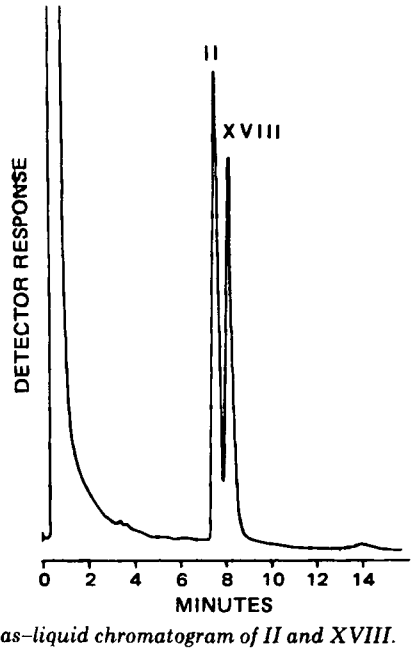


Figure 2—*Gas–liquid chromatogram of II and XVIII.*

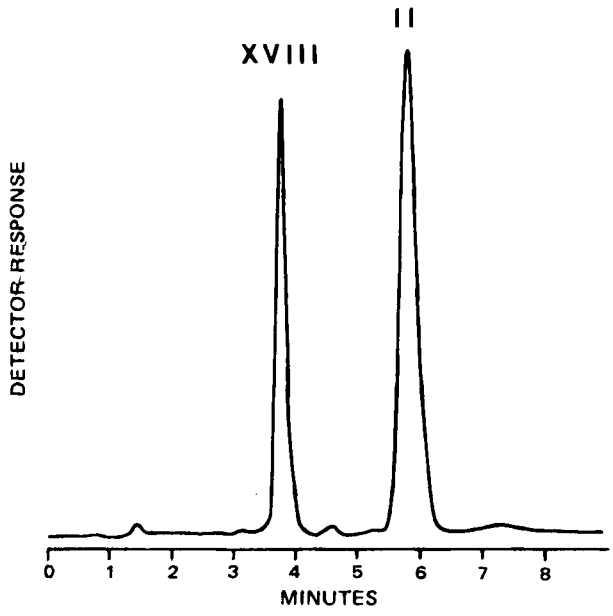


Figure 3—*High-pressure liquid chromatogram of II and XVIII.*

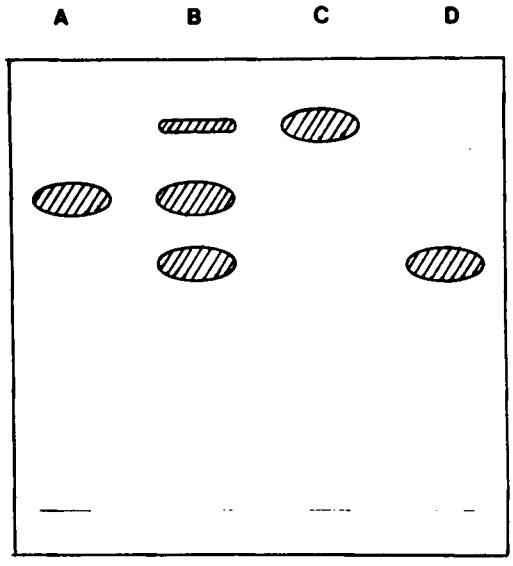


Figure 4—*Schematic representation of the TLC separation of IV, XVII, and XVIII. Key: A, XVII; B, urine band with* R_f *equal to that of V and XV eluted from the plate and treated with diazomethane and then acetic anhydride; C, XVIII; and D, IV.*

over sodium sulfate and concentrated to about 1 ml. The blanks were obtained according to the same procedure.

TLC—The chloroform extract (0.3 ml of the concentrated solution) was deposited on silica gel F-254 layers using benzene–dioxane–acetic acid (90:10:2, v/v/v) as the eluent and appropriate blanks and authentic samples for comparison.

TLC spots other than those common to the blank were scraped off and extracted with methanol. A UV spectrum was recorded for each solution, which was evaporated subsequently to dryness and then treated with an ether solution of diazomethane. The methyl esters were acetylated at 40° over 2 hr with pyridine and acetic anhydride, the excess of which was destroyed with methanol.

Derivatives IV, IX, XVII, and XVIII thus were obtained from V, X, XV[3], and XVI, respectively. These derivatives were analyzed for homogeneity by GLC, TLC, and high-pressure liquid chromatography (HPLC). Final identification was by mass spectrometry, with the appropriate inlet (direct or GLC), through comparison with the corresponding authentic sample.

Derivatives IV, XVII, and XVIII also were separated by TLC using a 0.75% solution of ethanol in chloroform as the eluent.

GLC—Compounds II and XVIII were separated using a steel column (2 m × 2.5 mm i.d.), packed with SP-1000 (3%) on Chromosorb HP (80–100 mesh) at 190°, with nitrogen as the carrier gas (30 ml/min). An E-301 (3%) on Chromosorb G (80–100 mesh) packing was used for IV and IX at 170°.

HPLC—Separations were performed with a steel column (25 × 0.26 cm) packed with Cyano-Sil-X-1 and set at 40° with a UV detector (285 nm). Chloroform–hexane (1:4, v/v, 1 ml/min) was the eluent.

Mass Spectrometry—Electron-impact mass spectra at 70 ev (source temperature 270–290°) were recorded *via* direct inlet at room temperature. When suitable, previous GLC separation was performed using helium as the carrier gas and a Ryhage separator at 300°. The spectral characteristics were reported previously (9).

Pharmacological and Toxicological Evaluations—The activity of the metabolites was tested in the carrageenan edema test (10). Mouse LD_{50} was determined according to the Spearman–Kärber procedure (11). All metabolites were less active than I and had similar toxicity.

RESULTS AND DISCUSSION

The preliminary data obtained by examination of the thin-layer chromatogram of the urine of rats treated with I suggested the likely formation of metabolites with the same dihydrobenzofuranic structure as I but with higher polarity. However, the neat benzofuranic UV spectrum of the spot corresponding to I was unexpected. The high reactivity toward acetic anhydride of the more polar metabolites and other chemical evidence, as well as known pharmacokinetic transformations [*e.g.*, benzylic hydroxylation (12) and ω and $\omega - 1$ side-chain hydroxylations (13)], indicated that the more polar metabolites were hydroxylated, nonphenolic compounds.

The puzzle of the benzofuranic spot was resolved upon observation of its variable intensity in different extraction conditions of the same urine pool. In particular, a nearly complete disappearance of the benzofuranic spot, accompanied by a slight increase in intensity of one of the polar spots, was observed under strictly defined extraction conditions. This observation suggested that it was a possible chemical artifact due to the decomposition of one of the actual metabolites to the benzofuranic analog of I. The following procedure was followed specifically for the identification of metabolites.

Chloroform extract A of rat urine was resolved into three TLC spots (a, b, and c in Fig. 1) which were not present in the chromatograms of the corresponding blanks. Spot a consisted of two components that could be separated and identified by GLC (Fig. 2) and HPLC (Fig. 3) of the corresponding methyl esters by comparison of their behavior with that of the analogous derivatives of authentic specimens of I and XVI. Spot b was resolved after the methylation–acetylation procedure into two spots (Fig. 4) with R_f values identical to those of independently prepared XVII and IV. Mass spectral analysis confirmed that spot b consisted of XV and

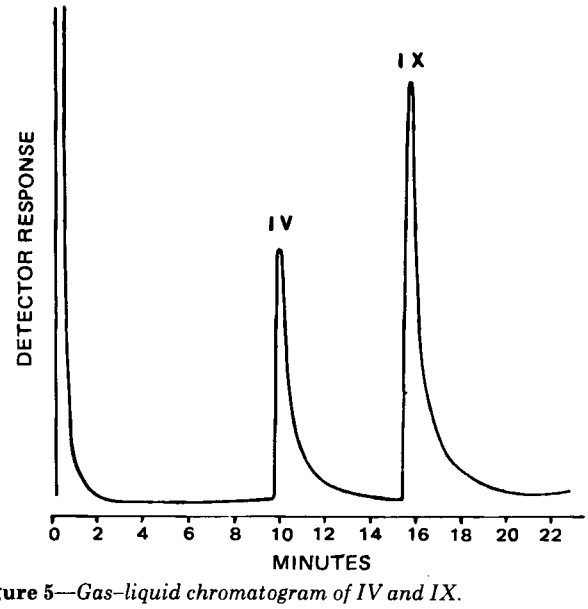


Figure 5—*Gas–liquid chromatogram of IV and IX.*

[3] On methylation and acylation, XV gave rise to XVII and to small amounts of XVIII.

Table I—Drug and Urinary Metabolites Separated from Different Animal Species [a]

Compound	Rats	Dogs	Humans
I	Trace	+	Trace
I glucuronide	−	+++	+++++
I labile acid derivative	−	+++++	++++
XV	+++	+	+
V	+++	+	−
X	++++	++	−

[a] The + indicates the relative abundance of the metabolites in the species.

V. Finally, spot c proved to be X on the basis of TLC and GLC data. The gas–liquid chromatogram of IV and IX is shown in Fig. 5.

In chloroform extracts B and C of rat urine, neither I nor compounds structurally related to I could be detected; a single spot with the same R_f as I was observed in the thin-layer chromatogram of the chloroform extracts B and C of both humans and dogs.

The mass spectra of the compounds extracted from the urine samples were identical to those of the derivatives of I, V, X, XV, and XVI. A comparative examination of the results obtained by the various techniques showed that rats metabolize I to the hydroxylated Compounds X, V, and XV.

Compound XVI is not a true metabolite, and its formation was due to a chemical artifact. In fact, it was only present in substantial amounts when the extraction was made in strongly acidic conditions, whereas the true Metabolite XV was detected in the extracts obtained in weakly acidic conditions. Compound XVI is formed from XV by dehydration.

In rat urine, traces of I also were present. In contrast to the findings for rats, the qualitative pattern of the metabolites of I in dogs was closer to that found in humans. Compound I was eliminated in a form conjugated with glucuronic acid, as shown by TLC of the extracts before and after treatment with β-glucuronidase.

Another conjugate, which gives I when treated with acid, was found in the urine of dogs and humans, but its identity was not elucidated. Amino acid conjugation is a possibility and was observed previously in the metabolism of other arylacetic anti-inflammatory drugs (14, 15). The results of the metabolism of I in different species are shown in Table I.

The initial stage of many biological oxidations is believed to be the abstraction of a hydrogen atom from the substrate. Lowering of the activation barrier for the homolytic cleavage of the CH bonds in I is expected only for the benzylic positions and the CH bond alpha to the ether oxygen. Interestingly, only the ring benzylic position was hydroxylated.

The toxicities of the metabolites were on the same order as the toxicity of the parent drug, and their activities in the carrageenan edema test were lower. Further detailed pharmacological and pharmacokinetic studies on I and its analogs are in progress in these laboratories.

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GLC Determination of α- and β-Tribenosides in Human Plasma

ANTOINE SIOUFI ˣ and FRANÇOISE POMMIER

Received July 6, 1979, from the *Biopharmaceutical Research Center, Ciba-Geigy, B.P. 308, 92506 Rueil-Malmaison, France.* Accepted for publication August 30, 1979.

Abstract □ The determination of α-tribenoside at concentrations down to 10 ng/ml and β-tribenoside at concentrations down to 5 ng/ml in human plasma is described. After addition of an internal standard, α- and β-tribenosides are extracted at basic pH into benzene. Both compounds are derivatized with *N*-heptafluorobutyrylimidazole. The derivatives are determined by GLC using a ^{63}Ni-electron-capture detector.

Keyphrases □ Tribenoside—GLC determination of α- and β-anomers, human plasma □ Anti-inflammatory agents—tribenoside, GLC determination of α- and β-anomers, human plasma □ Antiphlebotic agents—tribenoside, GLC determination of α- and β-anomers, human plasma

Tribenoside[1] (ethyl 3,5,6-tri-*O*-benzyl-D-glucofuranoside) is an anti-inflammatory and antiphlebotic agent. It is a mixture of about 33% of the α-anomer and 67% of the β-anomer.

No analytical technique is available for the quantitative assay of the two anomers in plasma. Tribenoside metabolism was studied in rats and dogs after administration of the ^{14}C-labeled drug (1); the blood concentration of the β-anomer remained higher than that of the α-anomer for the first few hours, but the situation was reversed after 20 hr. Metabolism splits the three benzyl groups and the ethyl group, giving several metabolites from each anomer.

This paper describes the GLC determination in plasma of α-tribenoside down to 10 ng/ml and of β-tribenoside down to 5 ng/ml, using β-clobenoside as the internal standard.

[1] Glyvenol, Ciba-Geigy.

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