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

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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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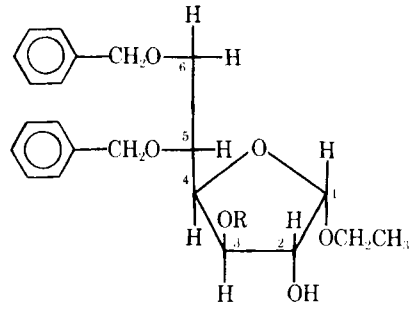
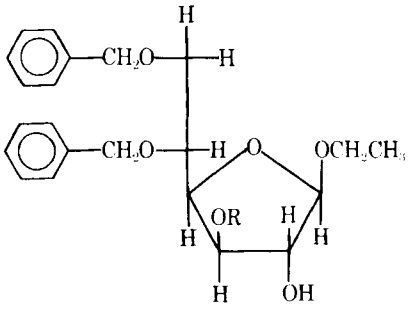
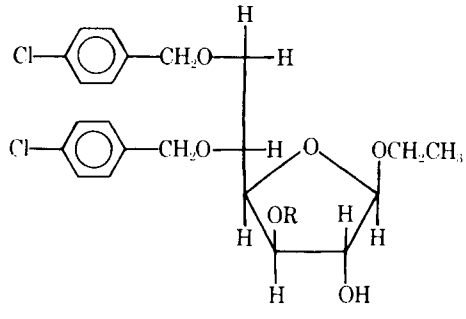


α-tribenoside: R = —CH_2—



β-tribenoside: R = —CH_2—



β-clobenoside: R = —C_7H_7

EXPERIMENTAL

Reagents—The benzene[2], sodium hydroxide[3], and heptane[3] were analytical grade. *N*-Heptafluorobutyrylimidazole[4] was stored at 4° in glass ampuls under nitrogen. The methanolic internal standard solution contained 500 ng of β-clobenoside[5]/ml.

Materials—The glassware was washed for 30 min in an ultrasonic bath, first with water and then with methanol.

The gas chromatograph[6] was equipped with a 15-mCi-^{63}Ni-electron-capture detector[7]. Peak areas were given by an electronic integrator[8]. The column was operated at 270°, the injector temperature was 300°, and the detector was set at 300° with argon–methane (90:10) at a 60-ml/min flow rate.

Glass columns (2 m × 3 mm i.d.) were washed with 1 *M* HCl, distilled water, acetone, and benzene and then were silanized with a 1% (v/v) solution of hexamethyldisilazane in benzene. The columns were washed again with benzene and dried at 100°.

The column packing[9] was 3% OV-17 on 100–120-mesh Gas Chrom Q. The filled columns were flushed with the carrier gas at a flow rate of 40 ml/min and heated to 300° at 1°/min. The column temperature was held overnight at 300° and at 270° throughout the next day. The temperature then was increased repeatedly from 150 to 250° for 24 hr. During these programmed cycles, the columns were conditioned further by the injection of 100 μl of a silylating agent[10] in portions between 150 and 220°. After this procedure, the columns were ready for use.

Extraction—The internal standard solution, 300 μl, was measured into a stoppered glass tube and dried under a nitrogen stream in a water bath at 37°. One milliliter of the sample, 2 ml of 0.1 *M* NaOH, and 4 ml of benzene were added. The tube was shaken mechanically[11] for 15 min and centrifuged at 5000 rpm for 10 min.

An aliquot of the benzene phase was transferred to another tube and dried under a nitrogen stream in a water bath at 37°.

Derivatization and Chromatography—To the dry residue were added 100 μl of heptane and 50 μl of *N*-heptafluorobutyrylimidazole. The solution was mixed thoroughly[12] for 30 sec and allowed to stand for 10 min at room temperature. Then 3 ml of water and 2 ml of heptane were added, and the tube was shaken thoroughly[12] for 30 sec and centrifuged for 3 min at 4000 rpm.

An aliquot of the heptane phase was transferred to another tube and dried under a nitrogen stream in a water bath at 37°; 500 μl of heptane was added, and the tube was shaken on a mixer[12].

[2] Mallinckrodt.
[3] Merck.
[4] Regis.
[5] Ciba-Geigy.
[6] Model 5710 A, Hewlett-Packard.
[7] Model 18713 A, Hewlett-Packard.
[8] Model 3380 A, Hewlett-Packard.
[9] Applied Science Laboratories.

Table I—Precision and Reproducibility of α-Tribenoside Assay (n = 6)

Concentration Added, ng/ml	Mean Concentration Found, ng/ml	95% Confidence Interval	Coefficient of Variation, *SD* %
10	10	8.5–11.5	12.2
25	25	23–27	5.9
50	49	47–51	4.2
75	77	74–80	3.2
100	100	94–106	4.8
150	150	145–154	2.9

Table II—Precision and Reproducibility of β-Tribenoside Assay (n = 6)

Concentration Added, ng/ml	Mean Concentration Found, ng/ml	95% Confidence Interval	Coefficient of Variation, *SD* %
5	4.8	4.4–5.3	8.9
10	9.8	8.2–11.4	13.2
25	26	25–27	3.2
50	50	49–51	2.3
75	75	74–76	1.2
100	100	97–102	2.3
150	150	148–152	1.1

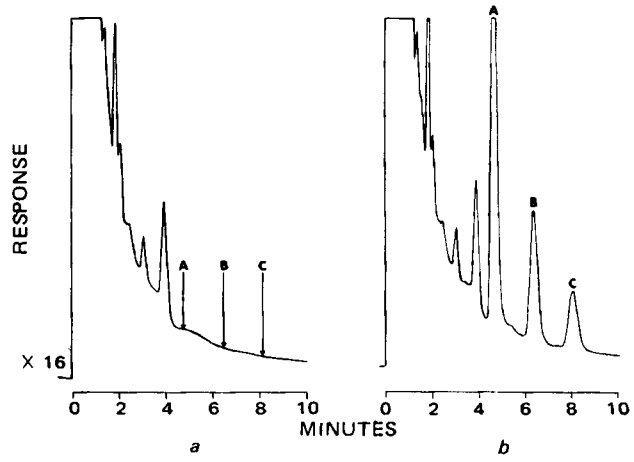


Figure 1—*Sample chromatograms of human plasma blank* (a) *and the same plasma spiked with 150 ng of internal standard/ml (A), 100 ng of β-tribenoside/ml (B), and 100 ng of α-tribenoside/ml (C)* (b).

[10] Silyl 8, Pierce.
[11] Infors.
[12] Vortex

Table III—α- and β-Tribenosides (Nanograms per Milliliter) in the Plasma of a Human Subject Given 800 mg of Tribenoside Orally for 7 Days

Anomer	Time after Administration								
	0 hr	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr	Day 2	Day 8
α-Tribenoside	ND[a]	41	53	20	10	6	6	11	14
β-Tribenoside	ND	94	92	51	36	24	24	52	47

[a] ND = not detected.

A 2-μl portion of the heptane solution was injected into the gas chromatograph by the solvent flush technique.

The α- and β-tribenoside content was calculated from the ratio of the peak areas by reference to a calibration curve prepared from a series of dried methanolic α- and β-tribenoside solutions to which plasma was added to yield concentrations between 2 and 150 ng/ml.

Human Subject—A healthy male subject, who had not received any drug for 8 days prior to the experiment and did not take any other drug during the study, received two 400-mg tribenoside capsules before breakfast for 7 days.

Blood samples were collected on the 1st day before and 1, 2, 3, 4, 6, and 8 hr after drug administration. Blood was collected on Days 2 and 3, just before the morning drug administration.

RESULTS AND DISCUSSION

Reaction Time—The derivatization was applied to 10, 25, and 500 ng of α- and β-tribenosides, and the reaction time was varied from 1 to 30 min. Derivative formation was evaluated by peak areas; derivatization was immediate, and the yield of the α- and β-tribenosides was the same over 1–30 min. For convenience and safety, the suggested derivatization time is 10 min.

Sensitivity and Precision—Tables I and II show that when the concentrations of α- and β-tribenosides reached the lower limits, the coefficient of variation increased to ~10. These lower concentrations (10 and 5 ng/ml for α- and β-tribenosides, respectively) may be taken as the assay sensitivity limits; lower concentrations could be detected but could not be determined accurately.

Plasma Interferences—Figures 1*a* and 1*b* show the chromatograms of a blank human plasma extract and the same plasma spiked with 100 ng of α- and β-tribenosides and 150 ng of β-clobenoside, respectively. No interference of the normal plasma components was noted.

Specificity—The four main metabolites (all a mixture of the corresponding α- and β-anomers) were derivatized under the same conditions as tribenoside. These metabolites were ethyl 3,5-di-*O*-benzyl-D-glucofuranoside, ethyl 3,6-di-*O*-benzyl-D-glucofuranoside, ethyl 5,6-di-*O*-benzyl-D-glucofuranoside, and 3,5,6-tri-*O*-benzyl-D-glucofuranoside. Their derivatives were not recorded when chromatographed under the same conditions as tribenoside at concentrations of ≤1 mg/ml.

Application—The technique was used to study the elimination of α- and β-tribenosides after daily oral administration to humans. The plasma concentrations of α- and β-tribenosides are given in Table III. The determinations were done in duplicate.

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Effect of Polyols on Interaction of Paraben Preservatives with Polysorbate 80

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Received April 3, 1979, from the *Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721.* Accepted for publication September 14, 1979.

Abstract □ A quantitative study of the interaction of the methyl, ethyl, propyl, and butyl esters of *p*-hydroxybenzoic acid with polysorbate 80 in the presence and absence of two polyols (propylene glycol and glycerol), which were potential competitors, was performed. The results indicate that neither competitor displaced significant amounts of the parabens from their binding sites on polysorbate 80. The previously observed synergistic antimicrobial effects of these polyols appear to be due to a mechanism other than the displacement of the parabens from their micellar binding sites.

Keyphrases □ Polyols—effect of propylene glycol and glycerol on interaction of parabens with polysorbate 80 □ Propylene glycol—effect on interaction of parabens with polysorbate 80 □ Glycerol—effect on interaction of parabens with polysorbate 80 □ Parabens—interaction with polysorbate 80, effect of propylene glycol and glycerol □ Preservatives—parabens, interaction with polysorbate 80, effect of propylene glycol and glycerol □ Polysorbate 80—interaction with paraben preservatives, effect of propylene glycol and glycerol

Several reports described the ability of nonionic surfactants (including polysorbates) to interfere with the activity of phenolic preservatives such as the parabens (1–4). This inactivation is believed to be due to the formation of a complex between the preservative molecules and the surfactant micelle, based on the assumption that the preservative antimicrobial activity is primarily due to the unbound form (3).

Previous work indicated that polyols such as propylene glycol or glycerol could function as preservatives or synergists to preservatives (5–7). Since these studies were designed to determine only whether preservative activity was enhanced by polyol addition, a systematic study was needed to evaluate the mechanism underlying the increased preservative activity associated with the polyols. Thus, a model system was developed to evaluate the ability

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