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Biotransformation of Phenprocoumon in the Rat†

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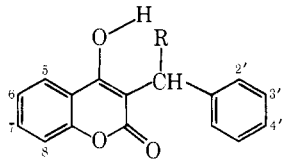
The metabolic fate of phenprocoumon [3-(α-ethylbenzyl)-4-hydroxycoumarin] in the rat is described. The major metabolites, 4′-, 6-, 7-, and 8-hydroxyphenprocoumon, have been identified by mass spectrometry, TLC, and uv and compared with authentic samples. Metabolites are mainly excreted via the feces. The results are compared with those previously reported for warfarin.

The biologic fate of the widely used oral anticoagulant, warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin, **1a**] has received considerable attention.[1] Recent results have demonstrated that the two enantiomeric forms of the drug are metabolized differently.[1f,g] The *S* isomer is stereoselectively oxidized to 7-hydroxywarfarin (7-OH-**1a**) and stereospecifically reduced to the *S,S* alcohol **1b**, while the R isomer is stereospecifically reduced to the *R,S* alcohol **1b**. Both isomers are oxidized to 6-hydroxywarfarin (6-OH-**1a**). Moreover, these metabolic pathways can be quantitatively affected to different degrees by prior administration of other drugs,[1g] e.g., phenylbutazone and secobarbital.[2] Since the potency of the two isomers is different, with the *S* isomer being approximately five times as active as the *R* isomer in both man[3] and the rat[4] and since the drug is clinically available as a racemic mixture, drug-induced differential quantitative changes in the routes of metabolism could account for some of the changes that are observed in pharmacological response.



1a, R = CH_2COCH_3
b, R = $CH_2CHOHCH_3$
c, R = CH_2CH_3

These findings have prompted us to investigate the metabolism of a closely related anticoagulant, phenprocoumon [3-(α-ethylbenzyl)-4-hydroxycoumarin, **1c**]. Like warfarin, the optical isomers of this drug also show widely different potencies and the absolute configuration of the most active isomer correlates with that of warfarin.[5] However, it differs from warfarin in that it is significantly more active, has a longer biologic half-life,[6] and reputedly gives a more stable and reliable hypoprothrombinemic response.[7]

A brief report on the excretion of phenprocoumon by the rat has appeared[8] and blood levels in man have been estimated.[9] As a prelude to future studies in man, we describe here the biotransformation of the drug in the rat and compare our results to those reported for warfarin.

Results

Excretion. The route and time course of the excretion of radioactive material from rats dosed with racemic [^{3}H]phenprocoumon were investigated in preliminary experiments. Within 4 days following tail-vein or intraperitoneal injection (6.9 mg/kg) approximately 17% of the administered radioactivity appeared in the urine and approximately 52% appeared in the feces. After 12 days, these levels rose to 20 and 59%, respectively. When a lower dose of phenprocoumon (0.4 mg/kg) was administered a similar excretion pattern was observed.

Further, in a sedated animal whose bile duct had been canulated, 18% of the administered dose appeared in the bile within 5.75 hr while less than 0.5% appeared in the urine.

Analysis of the Feces. MeOH extraction of powdered feces obtained during the first 4 days after dosing yielded 38% of the administered radioactivity. The residual fecal material retained some 10% of the original dose. On evaporation of the extract, the MeOH distillate contained less than 0.1% of the extracted radioactivity indicating a negligible amount of labile tritium in the extract. On distributing the residue between aqueous (pH ~11–12) base and Et_2O approximately 0.5% of labeled material remained in the Et_2O phase. After acidification of the aqueous phase, better than 97% of the radioactivity was recovered by subsequent Et_2O extraction.

The acidic materials were separated on preparative TLC using system 1 (Table I) into three fractions (hR_f, rel % radioactivity): A 0–1, 10%; B 1–30, 70%; C 30–54, 20%. Further chromatography of fraction B (three systems) yielded the major component whose chemical ionization (CI) mass spectrum contained significant ions at *m/e* 297, 179, 163,

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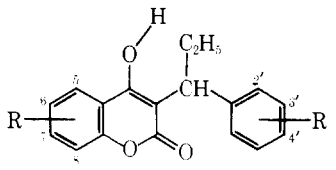
Table I. Thin-Layer Chromatography of Phenprocoumon Derivatives



hR_f

Solvent system[a]	H	5-OH	6-OH	7-OH	8-OH	2'-OH	3'-OH	4'-OH
1	37	7	5	7	24	23	12	8
2	55	15	9	10	27	28	19	17
3	31	18	22	11	17	29	25	20
4	48	22	28	28	35	44	33	33
5	50	24	32	34	47	47	34	32

[a]Eastman Kodak 6060 fluorescent silica gel sheets visualized by short-wave uv light: 1, $C_6H_5CH_3$-AcOH (9:1); 2, $CHCl_3$-AcOH (100:1); 3, *t*-BuOH-C_6H_6-NH_4OH-H_2O (45:20:9:3); 4, $CHCl_3$-EtOAc-AcOH (100:50:1); 5, $C_6H_5CH_3$-HCOOEt-HCOOH (10:5:1).

135, and 119 (Figure 1). These ions indicated the material contained two monohydroxylated derivatives of phenprocoumon, one bearing a hydroxy group on the coumarin ring (*m/e* 297, 179, 119) and the other having a hydroxyl group in the C-3 side chain (*m/e* 297, 163, 135).[10] Chromatographic separation of this mixture with system 4 gave two fractions, F_1, and F_2, corresponding to 4'- and 6-hydroxyphenprocoumon (4'-OH- and 6-OH-1c), respectively. CI mass spectrometry of F_1 gave ions at *m/e* 297, 163, and 135 and not at *m/e* 179 or 119 and thus confirmed that the position of hydroxylation was on the phenyl ring. CI mass spectrometry of F_2 gave ions at *m/e* 297, 179, and 119 and not at *m/e* 163 or 135 and thus confirmed that the position of hydroxylation was on the coumarin nucleus.[10] Accurate mass measurements using high-resolution electron impact (EI) mass spectrometry on the ions at *m/e* 296, 267, and 121 established the hydroxyphenyl structure of F_1 while mass measurements of the ions at *m/e* 297, 267, and 137 confirmed the dihydroxycoumarin structure of F_2.[‡] Fraction F_2 was clearly distinguished from 5-, 7-, and 8-hydroxyphenprocoumon by TLC (systems in Table I) and exhibited the distinctive uv spectrum of 6-hydroxyphenprocoumon.[10] Though 3'- and 4'-hydroxyphenprocoumon are not completely separated by the systems shown in Table I, when samples of fraction F_1 were cochromatographed with these compounds individually better than 90% of the radioactivity was found in the 4'-hydroxyphenprocoumon band.

Purfication of fraction C yielded as the major component F_3, which was chromatographically identical with phenprocoumon. High-resolution EI mass spectrometry, uv, and crystallization with unlabeled phenprocoumon (isotope dilution) confirmed this identification.

Fraction A was examined by TLC system 5, hR_f 11–19 designated F_x, and did not cochromatograph with any of the standard compounds. Identification of this fraction requires further investigation but unchanged chromatographic behavior following incubation with glusulase suggests that it does not contain sulfate or glucuronide conjugates.

The discovery that 7-hydroxyphenprocoumon decomposes on TLC with system 1[10] but not with systems 2 or 3 prompted reinvestigation of the crude acidic extract. Some 6% of the radioactivity was found to chromatograph (two dimensional) with added 7-hydroxyphenprocoumon, indicating that a small quantity of this compound is formed metabolically. In similar fashion, 8-hydroxyphenprocoumon was also detected in the feces.

The distribution of radioactive materials in the crude extract is shown in Table II. 6- and 4'-hydroxyphenprocoumon are the major metabolites.

Analysis of Urine. Urine samples lost little radioactivity on lyophilization. Ether extraction of an acidic solution of lyophilized urine yielded 90% of the radioactivity. Acid–base partition revealed the radioactivity was associated with acidic material. Preparative TLC of the crude extract (system 5) gave four major bands (hR_f, percent ^3H): I 0–14, 4%; II 14–30, 19%; III 30–46 53%; IV 46–63, 24%. Analytical TLC revealed that these bands contained the following components: I, U_x (major) not equivalent to any of the standards; II, U_x (major) and 6- and 7-hydroxyphenprocoumon (minor); III, 6- and 4'-hydroxyphenprocoumon (major), 7- and 8-hydroxyphenprocoumon (minor); IV, phenprocoumon (major) and 8-hydroxyphenprocoumon (minor). Further preparative TLC of band III yielded fractions U_1, U_2, U_3, and U_4 which were equivalent to 4'-, 6-, 7-, and 8-hydroxyphenprocoumon, respectively. High-resolution EI mass spectrometry of structurally critical ions (see Experimental Section) confirmed the identity of U_3 and U_4. Treatment of band I with glusulase revealed U_x to be a mixture containing conjugates of 4'-hydroxyphenprocoumon (14%) and 6- and 7-hydroxyphenprocoumon (17%), but the major component was not identified. It should be noted, however, that U_x cochromatographed with F_x on two-dimensional chromatography (systems 2 and 3) and therefore these materials may be structurally identical.

Table II. Relative Amounts of Metabolites Found in the Crude Urine and Feces Extracts[a]

Compound	Rel %[b] Feces	Urine
F_x	13	
U_x		12
6-Hydroxyphenprocoumon	30	29
7-Hydroxyphenprocoumon	6	11
8-Hydroxyphenprocoumon	3	9
4'-Hydroxyphenprocoumon	27	20
Phenprocoumon	17	18

[a]Estimated from the amount of radioactivity associated with each standard after chromatography (see Experimental Section for details). [b]Expressed as percentage of total counts per plate.

The distribution of radioactive material in the crude urine extract is shown in Table II. As in the feces 6- and 4'-hydroxyphenprocoumon are the major metabolites.

Analysis of the Bile. Et_2O extraction of acidified bile yielded 91% of the radioactivity. Chromatography showed this material did not contain phenprocoumon or its monohydroxy derivatives (hR_f 0 with system 2). After incubation of bile samples with glusulase, 90% of the radioactive material was found to be chromatographically equivalent to phenprocoumon. Similar results were found if the en-

‡When these compounds are studied by EI mass spectrometry structurally significant ions emerge from the fragmentation patterns as they did from the CI studies.[10] A detailed analysis of EI-induced fragmentation routes of these compounds based on exact mass measurements and defocused metastables will be presented along with fragmentation mechanisms based on specifically labeled (^{13}C and ^2H) analogs in a future report.

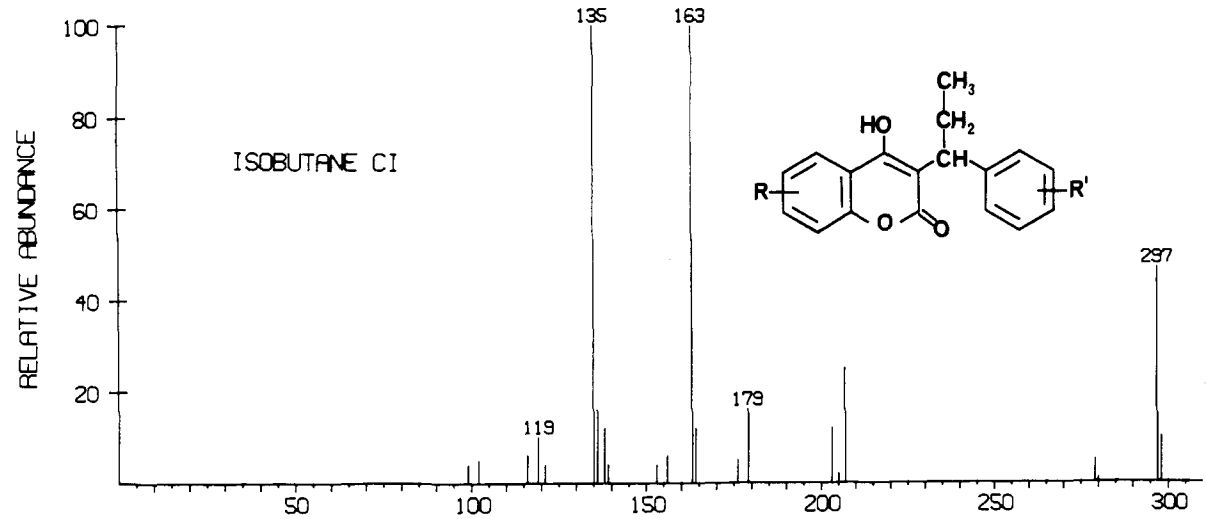


Figure 1. Isobutane CI mass spectrum of the major components of fraction B obtained from rat feces after TLC.

zyme was omitted from the incubation medium (pH 5, 37°, 72 hr).

Discussion

The finding that feces is the major route of excretion contrasts markedly with an earlier report of Goding and West.[8] These workers reported that following administration of ^{14}C (*R*)- and (*S*)-phenprocoumon separately to rats, radioactivity was excreted into the urine as 100 and 70% of the dose, respectively. The fact that they administered individual isomers rather than the racemate and utilized female rats[§] rather than male would seem to be insufficient to account for the differences observed. This discrepancy remains to be resolved.

Goding and West's findings for the fecal excretion of warfarin, 9% of either isomer after 150 hr, would also appear to be lower than that reported by other workers. Losito and Rousseau[11] found they could extract 9% of administered radioactivity in the feces within 4 days, whereas Berg[12] and Barker et al.[1a,13] report finding approximately 35% after 2 weeks.

Biliary excretion is a major factor in the clearance of both warfarin[14] and dicumarol.[15] In the case of warfarin, it has been demonstrated that the bile contains primarily hydroxylated metabolites rather than warfarin itself or a conjugated form of the drug.

Based on the amount of drug and metabolites recovered from feces in the present study, biliary excretion also appears to play a major role in the elimination of phenprocoumon. Unlike warfarin, however, the single rat study suggests that phenprocoumon is spilled into the bile as a sensitive acid-labile conjugate of phenprocoumon itself. A structural possibility for such a compound is the enol ether 4-*O*-glucuronide analogous to that isolated and reported as a metabolite of 4-hydroxycoumarin.[16] Such a metabolite might be expected to undergo hydrolysis in the gut[17] and result in enterohepatic recycling. If a similar mechanism were operative in man, it could provide an explanation for the difference in plasma half-life observed for phenprocoumon (160 hr)[9] and warfarin (36 hr).[3b]

The metabolites of phenprocoumon, 6-, 7-, 8-, and 4′-hydroxyphenprocoumon, are analogous to those reported for warfarin (Table III). The most striking features to emerge from these data are the excellent agreement found between our data and that reported by Ikeda,[18] despite the fact that they utilized induced microsomes, and the apparent reversal in the relative proportions of the 6- and 7-hydroxy metabolites formed both in vivo and in vitro. For warfarin, the amount of 7-hydroxy metabolite formed is at least double the amount of 6-hydroxy formed, whereas for phenprocoumon, the opposite situation obtains. The relative amounts of the 8-hydroxy and the 4′-hydroxy metabolites formed from the two parent drugs are approximately the same, at least in urine. Significant differences in the relative amounts of the 8-hydroxy metabolite produced by the microsomes, however, are apparent.

Ikeda et al.[18a] have demonstrated that in the metabolism of warfarin, the apparent K_m's for the formation of 6-, 7-, and 8-hydroxywarfarin are the same (1.5×10^{-4} mol) while their rates of formation are different. This suggests that perhaps the same microsomal enzyme or a closely associated enzyme complex is responsible for the generation of all three products. It further suggests that the dissociation of the enzyme substrate complex (ES) to enzyme and product is rate limiting, i.e., K_m is better represented as a dissociation constant K_s.

Given the similarity in structure between warfarin and phenprocoumon, it does not seem unreasonable to speculate that the same enzyme system is also responsible for the formation of 6-, 7-, and 8-hydroxyphenprocoumon. If this is indeed the case, the relative differences of metabolic products for the two substrates are intriguing and suggest that perhaps differences in the modes of binding of the substrate to the enzyme are responsible for changes in the activation energies leading to the different products. In terms of either resonance site activation for initial attack by ^{+}OH or epoxide formation followed by rearrangement, one would expect predominant formation of either the 6- and/or 8-hydroxylated isomers rather than the 7-hydroxylated product. Indeed, in model chemical systems such results have been obtained for warfarin.[18a]

Clearly, the system warrants and needs further investigation before any definite conclusions can be drawn. It is felt that chiral substrates such as warfarin and phenprocoumon, which are subject to aromatic hydroxylation at several positions are well suited for probing the steric and electronic events occurring during aromatic hydroxylation. Experiments which are designed to probe this system and which utilize the enantiomers (labeled with radioactive and stable isotopes) of both phenprocoumon and warfarin, as well as hydroxylated derivatives as potential enzyme inhibitors, are currently being explored.

[§]Species not reported.

Table III. Relative Amounts of the Hydroxylated Metabolites[a] of Warfarin and Phenprocoumon Obtained from the Feces, Urine, and Liver Microsomes of the Rat

	Feces		Urine		Microsomes		
	Warf[b]	Phen	Warf[c]	Phen	Warf[f]	Phen	Warf
6-OH		1	0.43	1	0.57	0.74	0.54
7-OH	Major	0.20	1	0.48	1	0.29	1
8-OH		0.10	0.26	0.31	0.18	0.61	0.18
4′-OH		0.90	0.60	0.69	[g]	1	0.71

[a]The data in the table are normalized with respect to the hydroxylated metabolites which occur in the largest quantity. [b]These data were obtained from ref 12 and 13. [c]These data were obtained from ref 1a. [d]These data were obtained from ref 18 and are the results from phenobarbital-induced animals. The quantity of 4′-hydroxywarfarin was not reported. [e]These data were recently obtained from our laboratory from a single source of microsomes and represent the average of two runs, each of which was run in duplicate. It is reported here to provide a comparison for the in vivo results. Details and experimental protocol along with individual isomer results will be reported in a future publication.

Experimental Section

Radioactivity was measured with a Packard Tri-Carb 3375 or a Beckmann LS 230 liquid scintillation counter in 10 ml of Aquasol (NEN). Counting efficiency was determined by internal standardization with [^{3}H]toluene (NEN). Radioactivity in the feces was determined by combustion of an aliquot in a Packard sample oxidizer. CI mass spectra were determined on a modified[19] AEI MS-902 high-resolution mass spectrometer using isobutane as the reagent gas. High-resolution mass measurements were determined in the EI mode on an AEI MS-9 high-resolution mass spectrometer by the electrical peak matching technique using perfluorotributylamine as standard. Glusulase (Endo) containing 134,000 units/ml of β-glucuronidase and 41,000 units/ml of aryl sulfatase was used for incubations. Fluorescent silica gel plates (Analtech) were used for preparative TLC and fluorescent silica gel sheets (Eastman Kodak 6060) were used for analytical TLC. Compounds were visualized under short-wave uv, 254 nm (UVS 11, Ultraviolet Products, Inc.). Radioactive loci on the TLC plates were determined using a Varian aerograph radio scanner Model LB 2722.

[^{3}H]Phenprocoumon. PtO_2, 75 mg, was placed in a 10-ml tube containing a stirring bar and 5 ml of H_2O. To this suspension was added $NaBH_4$, 200 mg,[20] in small portions with vigorous stirring. Upon completion of the addition, the suspension was heated to 70° to hydrolyze excess $NaBH_4$. The H_2O was removed and the Pt residue washed with 4 × 2 ml of H_2O. To the residue was added ^3H_2O, 1 ml, specific act. 1 Ci/ml (NEN), NaOH, 110 mg (2.75 m*M*), and phenprocoumon, 770 mg (2.75 m*M*). The tube was sealed, heated in an oil bath to 100°, and allowed to react for 9 hr with stirring. The reaction vessel was cooled and the contents were frozen in liquid N_2 prior to opening. The tube was opened and placed in a round-bottom flask. The flask was fitted with a connecting tube which was further mated to another connecting tube that had a side arm for connection to a vacuum pump. The second connecting tube was fitted with a receiver flask while its sidearm was connected to a (three-way) stopcock. The system was evacuated to 2 mm and closed and the pump was removed. The reaction vessel was warmed to room temperature while the receiver flask was cooled in liquid N_2. After the ^3H_2O had been transferred to the receiver flask, approximately 5 hr, the contents of the reaction vessel were extracted with NaOH (2 *N*) and filtered. The filtrate was acidified (concentrated HCl) and the precipitate was collected and washed several times with H_2O and then once with CH_2Cl_2. The aqueous filtrate was extracted with CH_2Cl_2 and the CH_2Cl_2 extract saved for subsequent work-up. The precipitate was recrystallized from aqueous EtOH, followed by C_6H_6–hexane and then aqueous EtOH. The recrystallized material was dissolved in 2 *N* NaOH, precipitated with acid (concentrated HCl), collected, washed, and recrystallized as above. The process was repeated three times and after a final recrystallization from aqueous EtOH it was collected and dried over P_2O_5 at 80° (2 mm) for 5 hr: yield 220 mg; specific act., 10 mCi/mmol. This material fluoresced as a single spot on TLC, system 1, 2, or 3 or after two-dimensional TLC on systems 2 and 3 (Table I), coincident with phenprocoumon while the TLC radio scanner indicated that the only radioactivity on the plate above background was associated with this spot.

Route of Excretion. [^{3}H]Phenprocoumon, 2.75 μCi/mg (obtained by dilution of the material reported above with cold phenprocoumon, in normal saline, pH 7), was administered to three groups of 475-g male Sprague–Dawley strain rats: group 1, four animals, 3.25 mg in 0.5 ml of solution per animal ip; group 2, five animals, 3.25 mg in 0.5 ml of solution per animal iv (tail-vein); group 3, five animals, 0.19 mg in 0.6 ml of solution per animal ip. Urine and feces were collected daily for 12 days. Urine within each group was bulked, diluted to 100 ml, and 0.1-ml samples were counted. Feces from each group were air-dried for 24 hr and powdered and 100–200-mg samples were processed in the sample oxidizer and counted.

After bile cannulation 3 mg of [^{3}H]phenprocoumon in 0.6 ml of solution was administered ip. Bile was collected in cooled containers for 5.75 hr, total volume 7.7 ml. Urine was obtained by syringe from the bladder. Anesthesia with pentobarbital (total 60 mg) was maintained throughout the experiment.

Isolation of Metabolites. Forty male Sprague–Dawley rats (350 g) were each injected ip with [^{3}H]phenprocoumon, 2.5 mg (6.9 μCi), in 0.5 ml of normal saline (adjusted to pH 7). Urine and feces were collected daily and frozen at −20° until analyzed.

(1) **Metabolites in the Feces.** Combined feces from the first 4 days were air-dried, powdered, and exhaustively extracted with cold MeOH. After evaporation under reduced pressure the residue was distributed between equal volumes of Et_2O and aqueous alkali. The aqueous alkali was washed with Et_2O, acidified, and extracted with Et_2O. Preparative TLC (system 1, Table I) gave three bands—A, hR_f 0–1; B, hR_f 1–30; C, hR_f 30–54—which were extracted with acetone. Band B was further purified by preparative TLC using system 5 (hR_f 35–50), system 3 (hR_f 34–42), and system 1 (hR_f 20–22). CIMS of this material gave ions, *m/e* (rel abundance) 297 (60), 179 (22), 163 (94) 135 (100), 119 (12). This material was separated with system 4 into two fractions, F_1 (hR_f 47) and F_2 (hR_f 40), and each fraction was rechromatographed with system 4 using 6- and 4′-hydroxyphenprocoumon as standards. F_1 corresponded to 4′-hydroxyphenprocoumon and gave the following ions by CIMS, *m/e* 297 (47), 163 (100) 135 (100), and by EIMS, *m/e* 296 ($C_{18}H_{16}O_4$), 267 ($C_{16}H_{11}O_4$) and 121 ($C_7H_5O_2$).‡ Samples of 3′- and 4′-hydroxyphenprocoumon were spiked with 7000 dpm of F_1 and chromatographed (Kodak) with system 3. In both cases ca. 5500 dpm (90%) was found in the 4′-hydroxy region and some 500 dpm (8.3%) in the 3′-hydroxyphenprocoumon region. F_2 corresponded to 6-hydroxyphenprocoumon and gave ions by CIMS, *m/e* 297 (100) 179 (27), 119 (18), and by EIMS, *m/e* 296 ($C_{18}H_{16}O_4$), 267 ($C_{16}H_{11}O_4$), 137 ($C_7H_5O_3$);‡ uv in ethanolic HCl, λ_{max} 332, 281 nm.

Band C was purified by preparative TLC using system 2 (hR_f 46–55), extracted with EtOH, rechromatographed using toluene–AcOH (100:1) and extracted with EtOH. Evaporation gave F_3, uv in ethanolic HCl λ_{max} 309, 284, 274 nm, after addition of NaOH: λ_{max} 313 nm; EIMS, *m/e* 280 ($C_{18}H_{16}O_3$), 121 ($C_7H_5O_2$), 119 (C_9H_{11}). Crystallization of 100 mg of phenprocoumon spiked with 10,600 dpm of F_3 from C_6H_6–hexane (twice) and EtOH–H_2O (four times) gave phenprocoumon 790 dpm/mg (75% of the radioactivity).

Band A chromatographed with system 5 showed radioactivity hR_f 11–19 and was designated F_x. This band, 7500 dpm, was incubated with 0.5 ml of glusulase in 10 ml of acetate buffer, pH 5.2, for 22 hr at 37°, acidified with HCl, and extracted with Et_2O. The Et_2O extract contained 5500 dpm. Another sample of A taken through the same procedures without glusulase gave 5800 dpm.

TLC of both materials showed radioactivity hR_f 0–3 (system 2), hR_f 11–19 (system 5).

(2) Metabolites in Urine. Urine was lyophilized and the residue taken up in aqueous H_3PO_4 and exhaustively extracted with Et_2O. After evaporation the extract was dissolved in aqueous NaOH, washed with Et_2O, acidified with HCl, reextracted into Et_2O, and evaporated under reduced pressure to yield ca. 8 g of dark oil containing 85% of the radioactivity in the urine. Preparative TLC using system 5 revealed four major bands—I, hR_f 0–14 (4% of the radioactivity); II, hR_f 14–30 (19%); III, hR_f 30–46 (53%); IV, hR_f 46–63 (24%)—which were extracted with acetone. Band III was further fractionated by preparative TLC with 5-, 6-, 7-, 8-, 2′-, 3′-, and 4′-hydroxyphenprocoumon as markers using successively system 2, 3, 2 (each plate developed ×3), and 3 to yield fractions U_1, U_2, U_3, and U_4 chromatographically equivalent to 4′-, 6-, 7-, and 8-hydroxyphenprocoumon, respectively: EIMS of U_3 and U_4 *m/e* 296 ($C_{18}H_{16}O_4$), 267 ($C_{16}H_{11}O_4$), 137 ($C_7H_5O_3$), and 119 (C_9H_{11}). Samples of band I were incubated at pH 5.2 both with and without glusulase as described for band A above. The Et_2O extracts were examined by TLC (system 2 developed ×2).

Samples of crude urine and feces extracts were cochromatographed with a mixture containing 5 μg each of phenprocoumon and 5-, 6-, 7-, 8-, 2′-, and 4′-hydroxyphenprocoumon using systems 2 and 3. Bands were located with uv light, cut out, and placed in liquid scintillation vials with 1 ml of EtOH, and 10 ml of Aquasol was added for liquid scintillation counting.

(3) Metabolites in the Bile. Samples of bile were acidified to pH 2 with H_3PO_4 and extracted with Et_2O. Further samples were incubated at 37°, 72 hr, with glusulase in acetate buffer, pH 5.2, acidified, and extracted with Et_2O. Controls were run without the enzyme. These extracts were examined by TLC in systems 2 and 4 and compared with standards.

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Notes

Potential Antimalarials. 9. Resolution of α-Diheptylaminomethyl-6-bromo-9-phenanthrenemethanol by an Unusual Method[1]

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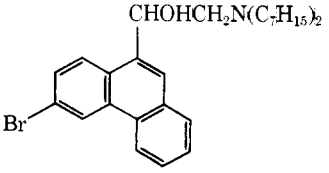
The titled base was resolved by crystallization of its *d*-tartrate salt from a solution of about 40% *d*-tartaric acid in aqueous methanol, a solvent system which might be called a chiral solution.

The resolution of the title compound, known as the May compound, proved to be a difficult task because of the soft, waxy, almost noncrystalline nature of its organic acid salts.



All standard methods failed. The resolution finally was accomplished by employing a very large excess of the resolving agent, in this case, *d*-tartaric acid, in a partially aqueous methanol solution. One might consider this separation a resolution from a chiral solution, a technique which apparently has not been employed previously.[3] However, Buchanan and Graham[4] have resolved optically unstable antipodes by crystallization from the optically active solvent, (+)-ethyl tartrate. This difference in solubility of the antipodes in (+)-ethyl tartrate has been explained theoreti-