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Synthesis and Thin-Layer Chromatographic, Ultraviolet, and Mass Spectral Properties of the Anticoagulant Phenprocoumon and Its Monohydroxylated Derivatives†

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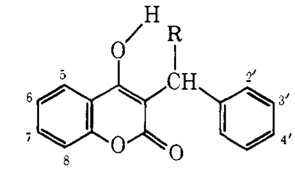
Phenprocoumon and all of its aromatic monohydroxylated derivatives have been synthesized and analyzed by TLC, uv, and chemical ionization mass spectroscopy. By utilization of various combinations of these analytical techniques all of the titled compounds can be uniquely identified.

As a continuation of our studies on the biotransformation of warfarin (1)[1] and the relationship between drug-induced interactions and its metabolism,[1d,2,3] we have begun a study of the metabolism of the closely related oral anticoagulant phenprocoumon (2).

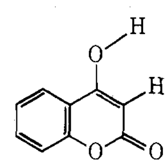
Although the two drugs are structurally quite similar, significant differences in their pharmacologic properties exist. For example, in an extensive clinical study phenprocoumon has been reported to elicit a more stable and reliable hypoprothrombinemic response compared to warfarin.[4] In addition, it is significantly more active than warfarin and has a much longer biologic half-life.[5] The reasons for these differences are at present obscure.

Since warfarin is known to be monohydroxylated in the 6, 7, 8, and 4′ positions in the rat[2,6] and in the 6 and 7 positions in man,[1,7,8] it seemed reasonable to anticipate that phenprocoumon would also be susceptible to aromatic hydroxylation. Indeed, preliminary work (mass spectrometry) in our laboratory had indicated the presence of such species in the urine of rats who had been injected with the drug. Hence, in order to facilitate the unambiguous identification of potential metabolites and to provide standards we embarked upon a synthetic program concurrent with our metabolic studies to characterize all the possible aromatic monohydroxylated derivatives of phenprocoumon.

Synthesis. Phenprocoumon (2) can be synthesized by alkylating 4-hydroxycoumarin (**3**) with 1-phenyl-1-propanol using HCl[9,10] or H_2SO_4[11] as catalysts or by using $POCl_3$[12] as a condensing agent. Alternatively, it can be synthesized by direct alkylation with 1-phenyl-1-bromopropane.[13] As a consequence, these reaction schemes were initially explored for the synthesis of 6-hydroxyphenprocoumon from 4,6-dihydroxycoumarin but were unsuccessful as only intractable tars were obtained. To circumvent the problem of O- vs. C-alkylation in the condensation reaction the 5-, 6-, 7-, and 8-hydroxy positions were initially protected with benzyl groups which were subsequently removed by hydrogenolysis.



1, R = CH_2COCH_3
2, R = CH_2CH_3



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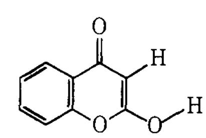

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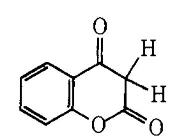

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A method similar to that of Hermodson, Barker, and Link[14] was utilized for the synthesis of 5-, 6-, 7-, and 8-benzyloxy-4-hydroxycoumarin and involved the selective‡ monobenzylation of the isomeric dihydroxyacetophenones. The acetophenones were then allowed to react with diethyl carbonate by the method of Dickenson[16] to yield the benzyloxy-4-hydroxycoumarins. Condensation of these materials with 1-phenylpropanol or 1-phenyl-1-bromopropane also led to intractable tars.

Since coumarin **3** can exist in two other tautomeric forms,[17] the chromone structure **4** and the diketo structure **5**, it seemed that alkylation of 4-hydroxycoumarin§ and its benzyloxy derivatives might be possible using conditions that are commonly employed for the alkylation of β-keto esters.[18] Sodium acetate was chosen as the base, since it was sufficiently strong to abstract the 4-hydroxy proton[19] but also weak enough to prevent significant dehydrobro-

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‡The ^1H NMR absorption of the intramolecular hydrogen-bonded ortho groups[15] at approximately 11 ppm clearly indicated the selectivity of benzylation.

§For a review of the reactions of 4-hydroxycoumarin, see Zanten and Nauta.[18]

Table I. Uv Spectral Data of Phenprocoumon and Derivatives[a]

Compound	Acidic ethanolic solution[b]	Basic ethanolic solution[b]
Phenprocoumon	244 (mn, 3.43), 274 (mx, 4.06), 278 (c), 285 (mx, 4.10), 293 (mn, 3.95), 300 (s), 310 (mx, 4.08), 320 (s)	240 (s), 265 (mn, 3.46), 295 (s), 314 (mx, 4.12)
5-Benzyloxy	232 (s), 254 (mn, 3.43), 299 (mx, 4.24), 308 (s), 325 (s)	250 (s), 271 (mn, 3.51), 316 (mx, 4.14)
5-Hydroxy	237 (s), 255 (mn, 3.38), 296 (mx, 4.21), 306 (s), 318 (s)	244 (s), 266 (mn, 3.50), 305 (s), 318 (mx, 4.13), 327 (s)
6-Benzyloxy	249 (mn, 3.57), 282 (mx, 4.14), 292 (s), 305 (mn, 3.66), 329 (mx, 3.94)	259 (mn, 3.36), 301 (mx, 4.12), 307 (c), 317 (mx, 4.13)
6-Hydroxy	248 (mn, 3.42), 281 (mx, 4.00), 290 (s), 306 (mn, 3.48), 333 (mx, 3.73)	269 (mn, 3.32), 305 (mx, 3.99), 345 (s, br)
7-Benzyloxy	254 (mn, 3.44), 278 (s), 287 (mx, 4.04), 292 (c), 316 (mx, 4.33), 325 (s)	243 (c), 248 (mx, 4.14), 254 (s), 270 (mn, 3.61), 290 (s), 313 (mx, 4.26)
7-Hydroxy	254 (mn, 3.35), 277 (s), 287 (mx, 3.90), 292 (c), 317 (mx, 4.22), 325 (s)	242 (c), 257 (mx, 4.13), 277 (mn, 3.55), 294 (s), 331 (mx, 4.31)
8-Benzyloxy	243 (s), 258 (mn, 3.75), 286 (mx, 4.18), 320 (s, br)	267 (mn, 3.58), 302 (mx, 4.10)
8-Hydroxy	240 (c), 244 (mx, 4.00), 262 (mn, 3.72), 290 (mx, 4.17)	255 (c), 258 (mx, 4.00), 265 (c), 269 (mx, 3.96), 278 (mn, 3.85), 305 (mx, 4.15)
2′-Hydroxy	243 (mn, 3.45), 274 (mx, 4.03), 279 (c), 284 (mx, 4.03), 293 (mn, 3.94), 302 (s), 311 (mx, 4.07), 322 (s)	262 (mn, 3.57), 281 (mx, 3.84), 286 (c), 295 (s), 315 (mx, 4.06)
3′-Benzyloxy	245 (mn, 3.47), 274 (mx, 4.09), 278 (c), 284 (mx, 4.09), 293 (mn, 3.95), 302 (s), 310 (mx, 4.08), 321 (s)	242 (s), 263 (mn, 3.56), 281 (mx, 3.83), 284 (c), 294 (s), 315 (mx, 4.12)
3′-Hydroxy[c]	245 (mn), 275 (mx), 278 (c), 284 (mx), 293 (mn), 302 (s), 310 (mx), 322 (s)	264 (mn), 296 (s), 314 (mx)
4′-Hydroxy	246 (mn, 3.49), 380 (mx, 4.07), 293 (mn, 3.97), 310 (mx, 4.09), 322 (s)	266 (mn, 3.62), 294 (s), 315 (mx, 4.09)

[a]The uv spectra were run on a Cary 14 spectrophotometer using 4-ml cuvettes with 1-cm path length and 3 ml of absolute ethanolic solution. The acidic spectra were obtained after the addition of 0.05 ml of HCl (0.1 *N*) to the ethanolic solution while the basic spectra were obtained after the addition of 0.1 ml of NaOH (2 *N*) to the acidic ethanolic solution. [b]mn = minimum; mx = maximum; s = shoulder; c = crest; absorptions are expressed in nanometers and absorbancies expressed as log absorbancy. [b]Absorbancies were not obtained for this compound since insufficient sample was available for an accurate weighing.

monation of 1-phenyl-1-bromopropane from occurring. Sodium acetate also served to trap the liberated HBr, thus inhibiting the possibility of debenzylation. The reactions were run at reflux in isopropyl alcohol and on work-up yielded 10–20% of the isomeric benzyloxyphenprocoumons[&] which upon subsequent hydrogenolysis yielded the corresponding hydroxylated phenprocoumons.

The synthesis of 2′- and 4′-hydroxyphenprocoumon was readily achieved by consensation of **3** with neat 1-(*o*-hydroxyphenyl)-1-propanol and 1-(*p*-hydroxyphenyl)-1-propanol, respectively. Similar condensations with 1-phenyl-1-propanol and 1-(*m*-hydroxyphenyl)-1-propanol could not be effected under similar conditions presumably because of the lack of resonance activation of the benzylic position. The synthesis of 3′-hydroxyphenprocoumon was ultimately accomplished in low yield by condensing **3** with 1-(*m*-benzyloxyphenyl)-1-propanol using HCl as catalyst[9,10] followed by slow hydrogenolysis.

^{1}H NMR analysis of the benzyloxy- and hydroxyphenprocoumons confirmed (lack of a signal for a vinylic proton and the presence of signals for the ethyl side chain and benzylic proton) that the coupling reactions had occurred between the 3 position of the 4-hydroxycoumarins and the benzylic position of 1-phenyl-1-bromopropane and the alcohols.

Uv Analysis. The uv absorption spectra in both acidic and basic ethanolic solution of phenprocoumon and its benzyloxy and hydroxy derivatives are tabulated in Table I. The uv spectra, in acidic ethanolic solution, of the benzyloxyphenprocoumons and their corresponding hydroxyphenprocoumons are nearly identical as expected. Thus, the spectra indicate the absence of reduction or cleavage of the coumarin ring during hydrogenolysis or work-up of the benzyloxy derivatives.

The spectra of phenprocoumon and 2′-hydroxy-, 3′-hydroxy-, and 4′-hydroxyphenprocoumon are nearly identical, showing characteristic maxima at approximately 275, 285, and 310 nm, in acidic ethanolic solution, and a maximum at approximately 315 nm, in basic ethanolic solution. The maxima at approximately 275 and 285 nm, in acidic ethanolic solution, are not completely resolved for 4′-hydroxyphenprocoumon, resulting in a maximum at 280 nm. The spectrum of 2′-hydroxyphenprocoumon contains an additional maximum at 281 nm, in basic ethanolic solution, which does distinguish it from phenprocoumon and 3′-hydroxy- and 4′-hydroxyphenprocoumon.

As expected, hydroxyl substitution in the coumarin ring leads to significant spectral changes, in both acidic and basic ethanolic solution. These differences allow 5-hydroxy-, 6-hydroxy-, 7-hydroxy-, and 8-hydroxyphenprocoumon to be distinguished one from the other and from phenprocoumon. The maxima at 281 and 333 nm in acidic ethanolic solution are clearly indicative of 6-hydroxyphenprocoumon, while maxima at 257 and 311 nm in basic ethanolic solution distinguish 7-hydroxyphenprocoumon. In like manner the maxima at 244 and 290 nm in acidic ethanolic solution are characteristic of 8-hydroxyphenprocoumon, whereas a maximum at 296 nm in acidic ethanolic solution is indicative of 5-hydroxyphenprocoumon.**

[&]O-Alkylated products were isolated from each condensation reaction mixture, idenified by ^{1}H NMR, and presumably account at least in part for the low yields obtained.

**Similar substituent effects have been found with warfarin and its hydroxylated derivatives. In fact, the spectra of warfarin and its hydroxylated derivatives closely correspond to the spectra of phenprocoumon and its corresponding hydroxylated derivatives.[1a]

Table II. Isobutane Chemical Ionization Mass Spectra of Phenprocoumon and Its Hydroxylated Derivatives[a]

	% abundance[b]								
Compound	297	281	219	203	179	163	135	121	119
Phenprocoumon		100		2		68			7
5-Hydroxyphenprocoumon	1.5				100				32
6-Hydroxyphenprocoumon	100		2		67				10.5
7-Hydroxyphenprocoumon	66		2		100				21
8-Hydroxyphenprocoumon	46		5		100				11
2′-Hydroxyphenprocoumon	10.5			9		44	13	100	
3′-Hydroxyphenprocoumon	100			6		86	2	2	
4′-Hydroxyphenprocoumon	81			22		100	94		

[a]Only the major ions (*m*/*e*) are listed in this table. [b]The % abundance was calculated as a percent of the base peak ion.

Chemical Ionization Mass Spectrometry. Chemical ionization is a rather recently developed technique[20] which we have found to be quite useful in drug metabolism studies.[20c] In a mildly acidic plasma, such as that produced when isobutane is used as the reagent gas, the spectra obtained are typically characterized by an intense MH^+ molecular ion accompanied by few or no fragment ions. The behavior of phenprocoumon and its monohydroxylated derivatives in an isobutane plasma is, however, somewhat unusual in that there is considerable fragmentation (Table II). This may be the result of the extremely stable product ions and neutral fragments produced upon rearrangement and fragmentation of the MH^+ molecular ions. Nevertheless, the spectra obtained using this technique, besides being interesting in themselves, are also useful in locating the position of hydroxyl substitution.

The observed parent and fragment ions for phenprocoumon and its monohydroxylated derivatives can be rationalized on the basis of four fragmentation routes (A-D, Scheme I). Fragmentation route A can result from protona-

Scheme I

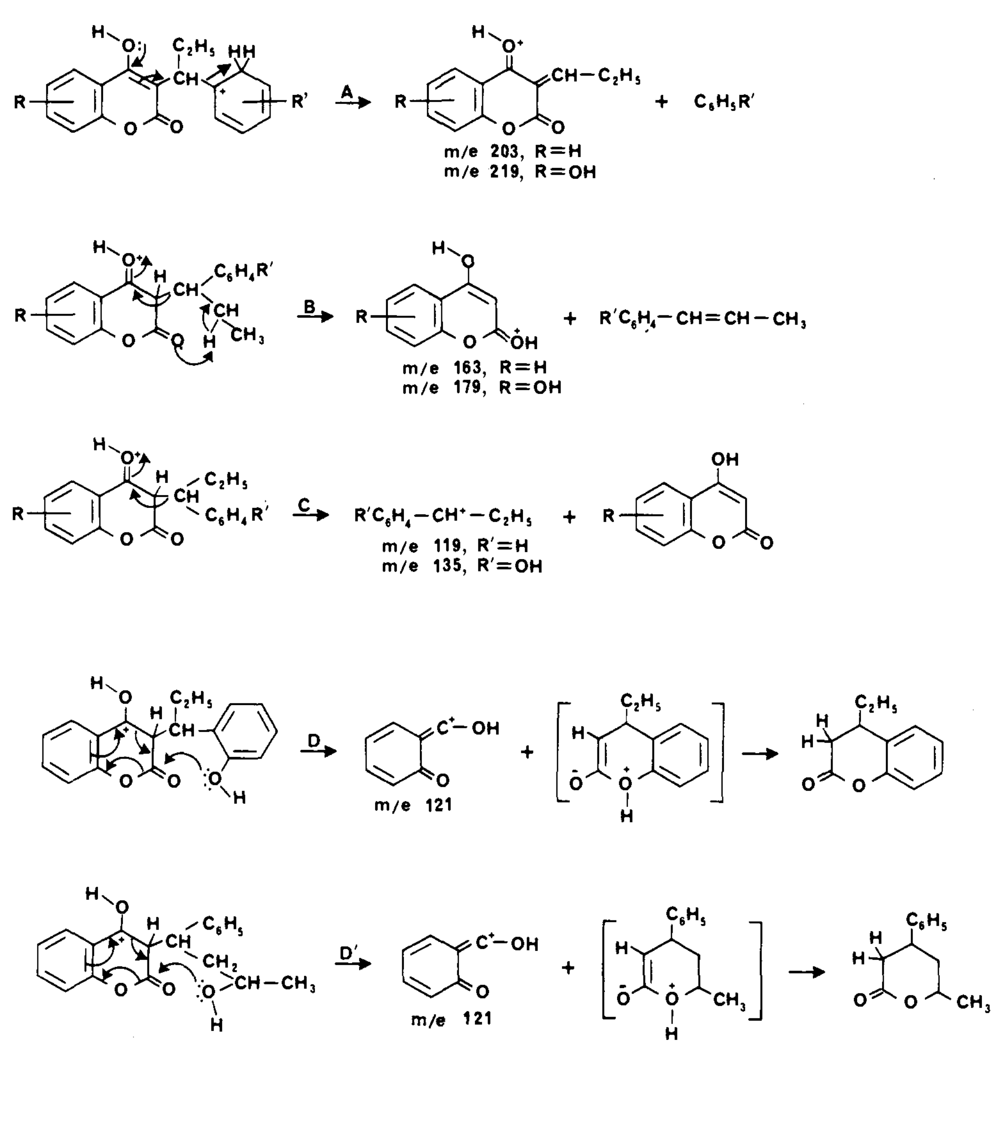

Table III. Thin-Layer Chromatography of Phenprocoumon and Derivatives[a]

Compound	Solvent system[b] 1	2	3	4	5	Fluorescence[c] at 254 nm
Phenprocoumon	37	55	31	48	50	Blue-violet
5-Hydroxyphenprocoumon	7	15	18	22	34	Light blue
6-Hydroxyphenprocoumon	5	9	22	28	32	Green-blue
7-Hydroxyphenprocoumon	7	10	11	28	34	Blue-violet
8-Hydroxyphenprocoumon	24	27	17	35	47	Blue-violet
2′-Hydroxyphenprocoumon	23	28	29	44	47	Black-brown
3′-Hydroxyphenprocoumon	12	19	25	33	34	Blue-violet
4′-Hydroxyphenprocoumon	8	17	20	33	32	Blue-violet

[a]TLC analyses were run on Eastman Kodak 6060, 100-μ, fluorescent silica gel plates using a uv (254 nm) lamp for visualization. Values are expressed as hR_f: distance traveled by compound per distance (16 cm) traveled by solvent front times 100. [b]1, toluene-acetic acid, 9:1; 2, $CHCl_3$-acetic acid, 100:1; 3, *tert*-butyl alcohol-benzene-NH_4OH-H_2O, 45:20:9:3; 4, $CHCl_3$-ethyl acetate-acetic acid, 100:50:1; 5, toluene-ethyl formate-formic acid, 10:5:1. [c]After exposure to NH_3 vapor, all fluorescent spots became more intense except 2′-hydroxyphenprocoumon which remained unchanged.

tion of the phenyl group in the side chain to give the MH^+ ion, followed by elimination of benzene or phenol and the formation of the fragment ions at m/e 203 in the case of phenprocoumon and the 2′-, 3′-, and 4′-hydroxy derivatives, or at m/e 219 in the case of 5-, 6-, 7-, and 8-hydroxyphenprocoumon. Fragmentation routes B and C can result from initial protonation at the 3 position in the coumarin nucleus. The resulting MH^+ ion can then rearrange and fragment via pathway B to give styrene and ions at m/e 163 for phenprocoumon and the 2′-, 3′-, 4′-hydroxy derivatives, or at m/e 179 for 5-, 6-, 7-, and 8-hydroxyphenprocoumon. The MH^+ ion can also decompose via pathway C to yield benzyl ions at m/e 119 for phenprocoumon and the hydroxylated coumarin ring derivatives, or at m/e 135 for hydroxylated phenyl ring derivatives.

These results (Table II) indicate that the phenyl side-chain hydroxylated derivatives of phenprocoumon can readily be distinguished from the coumarin ring hydroxylated derivatives. In addition, 5-hydroxyphenprocoumon can be differentiated from 6-, 7-, and 8-hydroxyphenprocoumon by the low intensity of its MH^+ ion at m/e 297 relative to the large intensity of the fragment ions at m/e 179 (pathway B) and at m/e 119 (pathway C), while 6-hydroxyphenprocoumon can be distinguished from the remaining two hydroxylated isomers by the ratio of its MH^+ ion at m/e 297 to the fragment ion at m/e 179 (pathway B). Furthermore, the results (Table II) indicate that 2′-hydroxyphenprocoumon can readily be distinguished from the 3′- and 4′-hydroxy isomers by the presence of an intense ion at m/e 121 (base peak). Moreover, 3′-hydroxyphenprocoumon can be differentiated from 4′-hydroxyphenprocoumon by the ratio of its MH^+ ion at m/e 297 to the fragment ion at m/e 163 (pathway C). The formation of the ion at m/e 121 for 2′-hydroxyphenprocoumon is deserving of further comment and can be explained by initial protonation at the 3 position in the coumarin nucleus, followed by rearrangement and fragmentation via pathway D. Support for this mechanism comes from the observation in our laboratory that the isobutane CI mass spectrum of warfarin alcohol (pathway D′) also contains a large ion at m/e 121. Thus, ideally all the ring monohydroxylated derivatives of phenprocoumon can be uniquely distinguished by this technique except 7- and 8-hydroxyphenprocoumon.

TLC Analysis. A minimum of 1–5 μg of phenprocoumon or its monohydroxylated derivatives had to be applied to a TLC plate in order to be visualized under a uv (254 nm) lamp. Several solvent systems were employed (Table III) in an attempt to separate a mixture of phenprocoumon and all its aromatic monohydroxylated derivatives into discrete fractions. Although one individual solvent system could not effect such a separation, two-dimensional chromatography utilizing systems 2 and 3 was successful.

7-Hydroxyphenprocoumon Decomposition Product. During the initial TLC analysis, it was observed that phenprocoumon and its hydroxylated derivatives appeared to turn yellow if they were allowed to remain on the TLC plates after development. Similar decomposition of warfarin and its hydroxylated derivatives has been observed in our laboratory and in the laboratory of other investigators.[21] Subsequently it was found that when 7-hydroxyphenprocoumon was developed in system 1, as opposed to system 2 or 3, and then analyzed by uv spectroscopy, nearly complete decomposition had occurred. In contrast, similar treatment of phenprocoumon or the other hydroxylated derivatives did not produce discernable decomposition.

The unique chemical reactivity of 7-hydroxyphenprocoumon on TLC with toluene–acetic acid warrants further investigation not only because of the interesting chemistry involved but because such unexpected reactivity could potentially lead to a serious source of error in any future quantitative studies involving this material or similar species.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were determined on Varian A-60 and T-60 nuclear magnetic resonance spectrometers, ir spectra on a Beckman 5A infrared spectrophotometer, uv spectra on a Cary 14 ultraviolet spectrophotometer, TLC on Eastman Kodak 6060 chromatographic plates, and EI MS on an AEI MS-9 high-resolution mass spectrometer at 70 eV. Exact mass measurements were determined within 15 ppm utilizing an on-line PDP-12 computer and a high-resolution program. In addition, many of the ions were measured manually to within 4 ppm utilizing the electrical peak matching technique with perfluorotributylamine as standard. Isobutane chemical ionization mass spectra were obtained using a specially constructed ion source. The ion exit slit was 0.125 by 0.001 in. while the electron entrance hole was 0.13 in. The electron gun voltage for CI was set at 510 V. The ion repeller for both EI and CI was variable between 0.0 and +1.0 V. The source and analyzer ion gauges read 4.2×10^{-4} and 1.2×10^{-5} Torr, respectively, with an isobutane ion source pressure of 0.4 Torr. Both sample and reagent gas were introduced via a specially designed direct insertion probe. All of the compounds studied were introduced into the mass spectrometer via the direct insertion probe and were vaporized at temperatures between 200 and 230°.

Exact mass measurements were made using an on-line computerized system employing a PDP-12 computer and perfluorokerosine as standard.

2-Hydroxy-4-benzyloxyacetophenone (**7**). A mixture of 2,4-dihydroxyacetophenone, 51 g (0.34 mol, Aldrich), benzyl chloride, 42.3 g (0.34 mol), KI, 5 g (0.03 mol), K_2CO_3, 50 g (0.36 mol), and acetone, 500 ml ($CaCl_2$ dried), was refluxed for 18 hr and then cooled to room temperature to yield a red suspension which was filtered; the filtrate was vacuum evaporated to give a light red solid residue which was recrystallized (MeOH, charcoal, Norit A) to yield **7**: 55 g (67%); pale yellow crystals; mp 101–102° (lit.[14] 105–106°; lit.[22] 104–104.5°); NMR (60 MHz, $CDCl_3$) δ (ppm) 11.23 (s, 1 H, exchanges with D_2O), 7.62 (d, 1 H, J = 9 Hz), 7.38 (s, 5 H), 6.50 (m, 2 H), 5.08 (s, 2 H), 2.50 (s, 3 H).

2-Hydroxy-5-benzyloxyacetophenone (**8**). Using the same method that was used in the preparation of compound **7**, 2,5-dihydroxyacetophenone (Aldrich) yielded a yellow solid which was recrystallized (MeOH, charcoal, Norit A) to give **8**: 55%; yellow crystals; mp 67–68° (lit.[14] 69–70°); NMR (60 MHz, $CDCl_3$) δ (ppm) 11.87 (s, 1 H, exchanges with D_2O), 7.38 (s, 5 H), 7.08 (m, 3 H), 5.02 (s, 2 H), 2.52 (s, 3 H).

2-Hydroxy-6-benzyloxyacetophenone (**9**). In like manner, 2,6-dihydroxyacetophenone (Aldrich) yielded a yellow solid which was recrystallized (MeOH, charcoal, Norit A) to give **9**: 40%; yellow crystals; mp 108–109°; NMR (60 MHz, $CDCl_3$) δ (ppm) 11.80 (s, 1 H, exchanges with D_2O), 7.45 (s, 5 H), 7.37 (t, 1 H, J = 9 Hz), 6.55 (m, 2 H), 5.18 (s, 2 H), 2.60 (s, 3 H).

2,3-Dihydroxyacetophenone (**10**). The method of Boehme and Scharpf[23] yielded **9**: 52%; yellow crystals; mp 95–96° (lit.[23] 98–98.5°); NMR (60 MHz, $CDCl_3$) δ (ppm) 11.07 (s, 1 H, exchanges with D_2O), 7.00 (m, 3 H), 5.96 (s, br, 1 H, exchanges with D_2O), 2.63 (s, 3 H).

2-Hydroxy-3-benzyloxyacetophenone (**11**). Using the same method that was used in the preparation of compound **7**, compound **10** yielded a brown oil which was recrystallized (MeOH, charcoal, Norit A) to give **11**: 40%; yellow crystals; mp 55–56° [lit.[14] bp 122–130° (0.05 mm)]; ir (KBr, cm^{-1}) 3100, 1635, 1575, 1445, 1360, 1310, 1290, 1250, 1025, 775; NMR (60 MHz, $CDCl_3$) δ (ppm) 11.17 (s, 1 H, exchanges with D_2O), 7.00 (m, 8 H), 5.15 (s, 2 H), 2.57 (s, 3 H).

4-Hydroxy-7-benzyloxycoumarin (**12**). Absolute EtOH (15 ml) in dry (Na) C_6H_6 (50 ml) was added dropwise, with mixing, to an ice-cooled suspension of NaH, 3.74 g (0.16 mol), and C_6H_6 (200 ml). This was followed by the addition of compound **7**, 19.36 g (0.08 mol), in C_6H_6 (300 ml). The reaction was then heated to boiling and solvent was slowly distilled off until the distillate temperature reached 80°. Dry (CaH_2) diethyl carbonate, 20 ml (0.10 mol), was then added and distillation was continued for 8 hr with occasional additions of C_6H_6 to maintain the volume at ca. 250 ml. The reaction mixture was then cooled to room temperature, H_2O (300 ml) was slowly added, and the resulting mixture was placed into a separatory funnel. The C_6H_6 layer was taken off, and the aqueous NaOH solution was extracted (Et_2O) and acidified (concentrated HCl) to give a precipitate which was filtered, washed (H_2O), and dried (vacuum desicator, P_2O_5) to yield **12**: beige solid (TLC pure); 16 g (76%); mp 272–274°; recrystallized (dimethoxyethane) to give pale yellow crystals; mp 273–274° (lit.[14] 272–273° from MeOH); NMR (60 MHz, DMSO-d_6) δ (ppm) 7.77 (d, 1 H, J = 10 Hz), 7.45 (s, br, 5 H), 7.04 (m, 2 H), 5.50 (s, 1 H, exchanges with D_2O, 3-vinylic proton), 5.23 (s, 2 H).

4-Hydroxy-6-benzyloxycoumarin (**13**). In a similar manner, compound **8** gave **13**: pale yellow solid (TLC pure); 60%; recrystallized (MeOH) to give pale yellow crystals; mp 225–226° (lit.[14] 230–231°); NMR (60 MHz, DMSO-d_6) δ (ppm) 7.41 (m, 8 H), 5.68 (s, 1 H), 5.20 (s, 2 H).

4-Hydroxy-5-benzyloxycoumarin (**14**). In like manner compound **9** yielded a solid residue, which was impure (TLC). The solid was triturated with warm $CHCl_3$ and filtered, and the filtrate was evaporated (steam bath) to give a solid, which was extracted (Et_2O) and recrystallized (Me_2CO–H_2O and then from MeOH) to yield **14**: 16%; pale yellow crystals; mp 135–140°; NMR (60 MHz, DMSO-d_6) δ (ppm) 7.52 (m, 6 H), 7.03 (m, 2 H), 5.62 (s, 1 H), 5.32 (s, 2 H).

4-Hydroxy-8-benzyloxycoumarin (**15**). Using the same procedure, compound **11** gave a light green solid which was recrystallized (MeOH) to yield **15**: 25%; light green crystals; mp 203–204° (lit.[14] 195–196°); NMR (60 MHz, DMSO-d_6) δ (ppm) 7.48 (m, 8 H), 5.73 (s, 1 H), 5.32 (s, 2 H).

1-Phenyl-1-bromopropane (**16**). Following the procedure of Pines and Schappell,[24] 1-phenyl-1-propanol (Chemical Samples Co.) yielded **15**: 85%; bp 91–92° (7 mm) [lit.[24] bp 89.5° (6 mm)]; NMR (60 MHz, $CDCl_3$) δ (ppm) 7.30 (m, 5 H), 4.83 (t, 1 H, J = 7 Hz), 2.22 (m, 2 H), 0.93 (t, 3 H, J = 7 Hz).

Phenprocoumon (**2**). Using a refined method of Schroeder, Titus, and Link,[13] 4-hydroxycoumarin, 20 g (0.12 mol, Aldrich), and compound **16**, 144 g (0.72 mol), were heated (155° with mixing for 1 hr) to give a pale red solution, which was taken up in NaOH (1 *N*) and extracted (Et_2O). The NaOH extract was then acidified (concentrated HCl) to give a white precipitate which was filtered and recrystallized (EtOH–H_2O) to yield **2**: 27 g (80%); white crystals; mp 174–175° (lit.[13] 175–177°); NMR (60 MHz, DMSO-d_6) δ (ppm) 8.13 (m, 1 H), 7.38 (m, 8 H), 4.48 (t, 1 H, J = 7 Hz), 2.27 (m, 2 H), 0.97 (t, 3 H, J = 7 Hz).

7-Benzyloxyphenprocoumon (**17**). A mixture of compound **12**, 5 g (20 mmol), and NaOAc, 16.4 g (200 mmol), was heated to reflux in isopropyl alcohol (300 ml). After 5 min, compound **16**, 39 g (200 mmol), was quickly added to the reaction mixture which was refluxed for an additional 4 hr to give a white suspension. Use of a large excess of **16** is necessary since a significant portion condenses with solvent to generate isopropyl 1-phenylpropyl ether. The reaction mixture was then filtered, the isopropyl alcohol was vacuum evaporated, and the resulting brown oil was taken up into NaOH (1 *N*), extracted (Et_2O), and acidified (HCl) to give a beige solid. The solid was filtered, washed (H_2O, petroleum ether), dried (vacuum desicator), dissolved in C_6H_6 (room temperature), filtered (to remove unreacted compound **12**), and recrystallized (C_6H_6) to yield **17**: 1.64 g (22%); white crystals; mp 150–151°; ir (KBr, cm^{-1}) 3100 (OH), 1650 (C=O); NMR (60 MHz, DMSO-d_6) δ (ppm) 7.98 (d, 1 H, J = 9 Hz), 7.23 (m, 12 H), 5.15 (s, 2 H), 4.36 (t, 1 H, J = 7 Hz), 2.15 (m, 2 H), 0.85 (t, 3 H, J = 7 Hz). Anal. ($C_{25}H_{22}O_4$) C, H.

Other solvents tried in this reaction included DMSO, dimethoxyethane, *t*-BuOH, and EtOH, but no increase in yield resulted.

6-Benzyloxyphenprocoumon (**18**). Using the same procedure, compound **13** yielded **18**: 13%; white crystals (from C_6H_6); mp 141–143°; ir (KBr, cm^{-1}) 3100 (OH), 1650 (C=O); NMR (60 MHz, $CDCl_3$) δ (ppm) 7.33 (m, 13 H), 5.05 (s, 2 H), 4.57 (t, 1 H, J = 7 Hz), 2.28 (m, 2 H), 1.02 (t, 3 H, J = 7 Hz). Anal. ($C_{25}H_{22}O_4$) C, H.

8-Benzyloxyphenprocoumon (**19**). In a similar manner, except refluxed for only 2 hr, compound **15** yielded **19**: 12%; white crystals (from CCl_4); mp 180–183°; ir (KBr, cm^{-1}) 3200 (OH), 1650 (C=O); NMR (60 MHz, $CDCl_3$ δ (ppm) 7.35 (m, 14 H), 5.22 (s, 2 H), 4.55 (t, 1 H, J = 7 Hz), 2.22 (m, 2 H), 1.05 (t, 3 H, J = 7 Hz). Anal. ($C_{25}H_{22}O_4$) C, H.

5-Benzyloxyphenprocoumon (**20**). A mixture of compound **14**, 500 mg (2 mmol), and NaOAc, 4.92 g (60 mmol), was heated to reflux in isopropyl alcohol (30 ml). After 5 min, compound **16**, 11.7 g (60 mmol), was quickly added to the refluxing reaction mixture. Three further additions of NaOAc, 1.64 g (20 mmol), and compound **16**, 3.9 g (20 mmol), were made after 14.5 hr, 16.5 hr, and 19 hr in order to react all the compound **14**. After 22 hr, the reaction was stopped to give a white suspension. The reaction mixture was then filtered, the isopropyl alcohol was vacuum evaporated, and the resulting oil was taken up into NaOH (1 *N*) and extracted (EtO_2). The EtO_2 extract was dried ($CaCl_2$) and then vacuum evaporated to give a yellow oil, which surprisingly contained the acidic compound **20**. Petroleum ether was added to the oil and the resulting suspension was placed into the freezer overnight, filtered, washed (petroleum ether) to yield a tinted yellow solid (a mixture of compound **20** and the O-alkylated product of compound **14**), which was dissolved in EtO_2, and extracted with NaOH. The NaOH extract was then acidified (HCl) to give a white solid which was extracted (into EtO_2), dried ($MgSO_4$), and vacuum evaporated to produce a white solid which was recrystallized (Me_2CO) to yield **20**: 14%; white crystals; mp 172–173°; ir (KBr, cm^{-1}) 3320 (OH), 1710 (C=O); NMR (60 MHz, $CDCl_3$) δ (ppm) 7.37 (m, 11 H), 6.77 (m, 2 H), 5.17 (s, 2 H), 4.30 (t, 1 H, J = 7 Hz), 2.23 (m, 2 H), 0.90 (t, 3 H, J = 7 Hz). Anal. ($C_{25}H_{22}O_4$) C, H.

1-(*m*-Hydroxyphenyl)-1-propanol (**21**). Using Auwer's procedure,[25] 3-hydroxybenzaldehyde (Aldrich) yielded **21**: 59%; yellow crystals (recrystallized from C_6H_6); mp 105–107° (lit.[25] 107°); NMR (60 MHz, DMSO-d_6) δ (ppm) 8.17 (s, 1 H, exchanges with D_2O), 6.83 (m, 4 H), 5.00 (br d, 1 H, J = 4 Hz, exchanges with D_2O), 4.35 (m, 1 H, a triplet results after D_2O exchange, J = 6 Hz, benzylic proton), 1.55 (m, 2 H), 0.80 (t, 3 H, J = 7 Hz).

1-(*m*-Benzyloxyphenyl)-1-propanol (**22**). The benzylation of compound **21** was run using the same conditions employed in the synthesis of compound **7**. After vacuum evaporation of the reaction mixture, the resulting yellow oil was dissolved in Et_2O, washed (NaOH, H_2O), dried ($MgSO_4$), and vacuum evaporated to yield **22**:

90%; pale yellow oil [lit.[26] bp 160–162° (0.5 mm)]; NMR (60 MHz, $CDCl_3$) δ (ppm) 7.32 (m, 9 H), 5.15 (s, 2 H), 4.68 (t, 1 H, J = 7 Hz), 1.80 (m, 2 H), 0.92 (t, 3 H, J = 7 Hz).

3′-Benzyloxyphenprocoumon (23). Dry HCl gas was bubbled into a stirred, warmed (40–50°) mixture of compound **22**, 12.1 g (5 mmol), 4-hydroxycoumarin, 10 g (6 mmol), and tetrachloroethane (125 ml). After 1.5 hr, the addition of HCl was stopped, and the thick reaction suspension was heated (140°) for an additional 3 hr, then cooled (room temperature), and filtered. The filtrate was then diluted with $CHCl_3$ and extracted with NaOH. The resulting NaOH extract was extracted (Et_2O), acidified (HCl), extracted (Et_2O), dried ($MgSO_4$), and vacuum evaporated to give a red oil, which was chromatographed on a silica gel column (Merck, 70–230 mesh) using C_6H_6 as the eluent (100-ml fractions were collected). Fractions 3–7 were combined and vacuum evaporated to give a pale yellow oil, which gave a white precipitate from a mixture of C_6H_6 and petroleum ether. The solid was recrystallized (MeOH–H_2O) to yield **23**: 1.1 g (6%); white needle-like crystals; mp 133–134°; ir (KBr, cm^{-1}) 3200 (OH), 1660 (C=O); NMR (60 MHz, $CDCl_3$) δ (ppm) 7.75 (m, 1 H), 7.30 (m, 12 H), 5.03 (s, 2 H), 4.53 (t, 1 H, J = 7 Hz), 2.17 (m, 2 H), 1.05 (t, 3 H, J = 7 Hz). Anal. ($C_{25}H_{22}O_4$) C, H.

7-Hydroxyphenprocoumon (24). A mixture of compound **17**, 1 g (2.6 mmol), 10% Pd/C (100 mg), and EtOH (90%, 100 ml) was shaken under H_2 (2 atm) for 16 hr. The reaction mixture was then filtered, and the filtrate vacuum evaporated to give a pale yellow oil, which was dissolved in Et_2O, dried ($MgSO_4$), and vacuum evaporated to yield **24**: 685 mg (98%); white solid; TLC pure; ir (KBr, cm^{-1}) 3215 (OH), 1665 (C=O); NMR (60 MHz, $CDCl_3$) δ (ppm) 7.67 (d, 1 H, J = 9 Hz), 7.42 (m, 5 H), 6.90 (m, 2 H), 4.47 (t, 1 H, J = 7 Hz), 2.22 (m, 2 H), 1.00 (t, 3 H, J = 7 Hz). A good analysis was not obtained since compound **24** could not be crystallized. High-resolution MS: found for $C_{18}H_{16}O_4$, 296.1062; calcd, 296.1049.

6-Hydroxyphenprocoumon (25). Using the same procedure, compound **18** gave a pale yellow oil which was recrystallized ($CHCl_3$) to yield **25**: 90%; pale yellow crystals; mp 81–83°; ir (KBr, cm^{-1}) 3200 (OH), 1650 (C=O); NMR (60 MHz, DMSO-d_6) δ (ppm) 7.25 (m, 8 H), 4.33 (t, 1 H, J = 7 Hz), 2.13 (m, 2 H), 0.83 (m, 3 H, J = 7 Hz). Anal. ($C_{18}H_{16}O_4$) C, H.

5-Hydroxyphenprocoumon (26). In the same manner, compound **20** gave a yellow oil which was recrystallized (EtOH–H_2O) to yield **26**: 70%; white crystals; mp 228–230°; ir (KBr, cm^{-1}) 3020 (OH), 1665 (C=O); NMR (60 MHz, Me_2CO-d_6) δ (ppm) 7.37 (m, 8 H), 4.45 (t, 1 H, J = 7 Hz), 2.20 (m, 2 H), 0.95 (t, 3 H, J = 7 Hz). Anal. ($C_{18}H_{16}O_4$) C, H.

8-Hydroxyphenprocoumon (27). Likewise, compound **19** yielded **27**: 57%; white solid; TLC pure; ir (KBr, cm^{-1}) 3300 (OH), 1660 (C=O); NMR (60 MHz, Me_2CO-d_6) δ (ppm) 7.37 (m, 8 H), 4.45 (t, 1 H, 7 Hz), 2.20 (m, 2 H), 0.95 (t, 3 H, J = 7 Hz). A good analysis was not obtained since compound **26** could not be crystallized. High-resolution MS: found for $C_{18}H_{16}O_4$, 296.1002; calcd, 296.1049.

3-Hydroxyphenprocoumon (28). A mixture of compound **23**, 800 mg (2.1 mmol), 10% Pd/C (80 mg), and EtOH (90%, 75 ml) was shaken under H_2 (2 atm) for 16 hr. TLC analysis indicated that the hydrogenolysis was approximately one-third complete. AcOH (7 ml) and 10% Pd/C (280 mg) were added to the reaction mixture, which was allowed to react for an additional 7 days at which time starting material could no longer be visualized on TLC. The reaction mixture was then filtered and the filtrate vacuum evaporated to give a dark red oil, which was chromatographed on a silica gel column (Merck, 70–230 mesh) using C_6H_6 as the eluent, to yield a fraction corresponding to **28**: 60 mg (10%); pale yellow oil; TLC pure; NMR (60 MHz, $CDCl_3$) δ (ppm) 7.73 (m, 1 H), 7.12 (m, 7 H), 4.45 (t, 1 H, J = 7 Hz), 2.15 (m, 2 H), 0.97 (t, 3 H, J = 7 Hz). A good analysis was not obtained since compound **28** failed to crystallize. High-resolution MS: found for $C_{18}H_{16}O_4$, 296.1040; calcd, 296.1049.

1-(*p*-Hydroxyphenyl)-1-propanol (29). A mixture of *p*-hydroxypropiophenone, 50 g (0.33 mol, Aldrich), and $NaBH_4$, 12.5 g (0.33 mol), was dissolved in NaOH (0.5 *N*, 800 ml) and stirred at room temperature for 24 hr. The resulting yellow solution was cooled in an ice bath and NH_4Cl, 129 g (3 mol), was slowly added to give a white precipitate which was allowed to stand overnight at room temperature (to permit the escape of NH_3), followed by cooling in the refrigerator, filtration, washing (cold H_2O), and air drying to yield **29**: 38.5 g (77%); white solid; mp 43–45°; NMR (60 MHz, CD_3CN) δ (ppm) 7.12 (m, 2 H), 6.72 (m, 2 H), 4.38 (t, 1 H, J = 7 Hz), 3.70 (s, br, 2 H), 1.52 (m, 2 H), 0.75 (t, 3 H, J = 7 Hz).

1-(*o*-Hydroxyphenyl)-1-propanol (30). In the same manner, *o*-hydroxypropiophenone (Aldrich) gave an emulsion after workup with NH_4Cl. The emulsion was extracted (Et_2O), and the Et_2O extract was dried ($MgSO_4$) and vacuum evaporated to yield **30**: 68%; pale yellow oil [lit.[27] bp 125–130° (0.25 mm)]; NMR (60 MHz, $CDCl_3$) δ (ppm) 8.30 (s, 1 H, br, exchanges with D_2O), 6.90 (m, 4 H), 4.60 (t, 1 H, J = 7 Hz), 3.93 (s, 1 H, br, exchanges with D_2O), 1.77 (m, 2 H), 0.85 (t, 3 H, J = 7 Hz).

4′-Hydroxyphenprocoumon (31). A mixture of 4-hydroxycoumarin, 6 g (37 mmol), and compound **29**, 20 g (132 mmol), was heated (100°) with stirring for 1.5 hr. The resulting pale green viscous dispersion was dissolved in Et_2O and extracted several times with a saturated $NaHCO_3$ solution. The aqueous extract was then acidified (HCl) to give a pale pink precipitate which was filtered, washed (H_2O), and recrystallized (EtOH–H_2O) to yield **31**: 4.1 g (37%); white crystals; mp 162–163°; ir (KBr, cm^{-1}) 3200 (OH), 1660 (C=O); NMR (60 MHz, pyridine-d_6) δ (ppm) 9.57 (s, 2 H), 8.17 (m, 1 H), 7.40 (m, 8 H), 4.78 (t, 1 H, J = 8 Hz), 2.50 (m, 2 H), 0.98 (t, 3 H, J = 7 Hz). Anal. ($C_{18}H_{16}O_4$) C, H.

2′-Hydroxyphenprocoumon (32). Using the same procedure, 4-hydroxycoumarin and compound **30** gave a white solid which was recrystallized ($CHCl_3$) to yield **32**: 31%; white crystals; mp 208–209°; ir (KBr, cm^{-1}) 3100 (OH), 1650 (C=O); NMR (60 MHz, DMSO-d_6) δ (ppm) 7.98 (m, 2 H), 7.13 (m, 7 H), 4.58 (t, 1 H, J = 7 Hz), 2.17 (m, 2 H), 0.97 (t, 3 H, J = 7 Hz). Anal. ($C_{18}H_{16}O_4$) C, H.

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Biotransformation of Phenprocoumon in the Rat†

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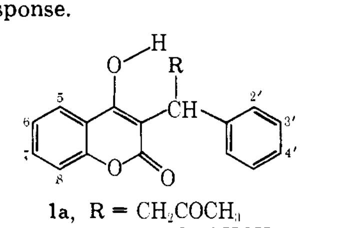
The metabolic fate of phenprocoumon [3-(α-ethylbenzyl)-4-hydroxycoumarin] in the rat is described. The major metabolites, 4′-, 6-, 7-, and 8-hydroxyphenprocoumon, have been identified by mass spectrometry, TLC, and uv and compared with authentic samples. Metabolites are mainly excreted via the feces.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