(15) C. D. Hurd, C. M. Buess, and L. Bauer, *J. Org. Chem.*, **17**, 865 (1952).
(16) Yields vary from 5 to 15%. In view of the highly successful preparation of **2** by Washburne's procedure,[9] no attempts were made to improve the yields by this method. Compound **2** prepared by this route is identical in all respects (ir, NMR, uv, melting point, and TLC properties) with that prepared by Rinkes' method[8] or Washburne's procedure.[9]
(17) U. Niedballa and H. Vorbruggen, *Angew. Chem., Int. Ed. Engl.*, **9**, 461 (1970); H. Vorbruggen and U. Niedballa, *Trans. N.Y. Acad. Sci.*, **36**, 705 (1974); U. Niedballa and H. Vorbruggen, *J. Org. Chem.*, **39**, 3654 (1974); **39**, 3660 (1974); **39**, 3672 (1974), and references cited therein.
(18) T. Ogawa, M. Yasui, and M. Matsui, *Agric. Biol. Chem.*, **36**, 913 (1972).
(19) E. H. Hamamura, K. Sato, and J. G. Moffatt, *J. Med. Chem.*, **15**, 1061 (1972).
(20) For some comments on the possible mechanism of the condensation of protected sugars with trimethylsilylpyrimidine bases in the presence of a Friedel–Crafts catalyst, see K. A. Watanabe, D. H. Hollenberg, and J. J. Fox, *J. Carbohydr. Nucleosides Nucleotides*, 1, 1 (1974).
(21) R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbruggen, *J. Am. Chem. Soc.*, **88**, 852 (1966).
(22) T. B. Windholz and D. B. R. Johnston, *Tetrahedron Lett.*, 2555 (1967).
(23) R. Barker and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 4605 (1961).
(24) This general acetolysis method was described by K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **33**, 1783 (1968).
(25) Under the same conditions, the reaction of the silylated derivative of **2** and the protected 1-methoxy sugar gives **4c** in low yield as evidenced by TLC.
(26) Cook reported removal of 2,2,2-trichloroethoxycarbonyl groups by zinc–copper couple: see A. F. Cook, *J. Org. Chem.*, **33**, 3589 (1968).
(27) Controlled potential electrolysis has also been used to remove the 2,2,2-trichloroethoxycarbonyl groups: see M. F. Semmelhack and G. E. Heinsohn, *J. Am. Chem. Soc.*, **94**, 5139 (1972); E. Kasafirek, *Tetrahedron Lett.*, 2021 (1972). We wish to thank Professor Dennis H. Evans of the Department of Chemistry, University of Wisconsin, for his help with the electrolytic experiments.
(28) L. B. Townsend, "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 2, W. W. Zorbach and R. S. Tipson, Ed., Wiley-Interscience, New York, N.Y., 1973, p 267.
(29) See ref 28, p 177.
(30) B. L. Currie, R. K. Robins, and M. J. Robins, *J. Heterocycl. Chem.*, **7**, 323 (1970), and references cited therein.
(31) The methods used for testing compounds in these cell culture systems have been previously described: see M. Umeda and C. Heidelberger, *Cancer Res.*, **28**, 2529 (1968).
(32) Trimethylsilyl azide was prepared by the method of S. S. Washburne and W. R. Peterson, Jr., *J. Organometal. Chem.*, **33**, 153 (1971).
(33) Although all available spectroscopic data are fully consistent with the assigned structures, no satisfactory analytical results could be obtained under the conditions used for the elemental analysis. However, purification by preparative TLC on silica gel with 10% acetic acid added to the developing solvent mixtures (for **4b**, 1:1 acetone–cyclohexane with 10% acetic acid; for **4c**, 2:1 carbon tetrachloride–ethyl ether with 10% acetic acid) gave satisfactory analysis with the inclusion of 2 mol of acetic acid for **4b** and 1.5 mol of acetic acid for **4c**. The inclusion of acetic acid in the samples purified by TLC is substantiated by the appearance of additional NMR resonances around δ 2.1, characteristic for the methyl group in acetic acid.
(34) M. Umeda and C. Heidelberger, *Proc. Soc. Exp. Biol. Med.*, **130**, 24 (1969).

Metabolic Formation and Synthesis of 1-(3-Chlorophenyl)-2-(4-hydroxyphenyl)-1-methyl-2-(2-pyridine)ethanol. A Potential Hypocholesteremic Agent

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1-(3-Chlorophenyl)-2-(4-hydroxyphenyl)-1-methyl-2-(2-pyridine)ethanol (**8a**) has been synthesized and found to be the major urinary metabolite following intraperitoneal administration of 1-(3-chlorophenyl)-1-methyl-2-phenyl-2-(2-pyridine)ethanol (1) to rats. This metabolite has a hypocholesteremic effect in rats similar to that of the parent drug.

The higher melting of two enantiomeric pairs of compound 1 was selected from a series of 133 2-(2-pyridine)-1,2-diarylalkanols for study of its hypocholesteremic effect in humans.[1] Although the compound was nontoxic and was found to lower serum cholesterol in rats, it had no hypocholesteremic effect in monkeys or man. This species difference in activity prompted the present investigation of the metabolism of compound 1 on the premise that a metabolite was responsible for hypocholesteremic activity. Compound 1 has been prepared with tritium uniformly distributed in the unsubstituted benzene ring.[2] This report describes characterization of the principal metabolites following ip injection into male Sprague–Dawley rats, synthesis of the metabolites, and preliminary evaluation of their hypocholestermic activity.

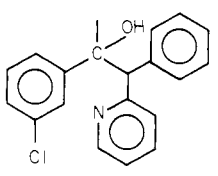

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Figure 1 shows the daily rate and cumulative urinary excretion for 13 days of three rats which had been administered 40 mg/kg (1.71 μCi) of compound 1. The first day's urine contained 5% of the original ^{3}H activity which decreased to less than 1% in 7 days. Excreted ^{3}H in feces followed a pattern similar to that of the urine with about 7% of the total dose excreted in the first day falling in 13 days to less than 1% per day. Total activity excreted in the feces averaged about 25% of the dose over this period. There was no appreciable ^{3}H detected in any of the 11 tissues examined after sacrifice. Total activity retained in body tissues was estimated to be less than 1.4%. The

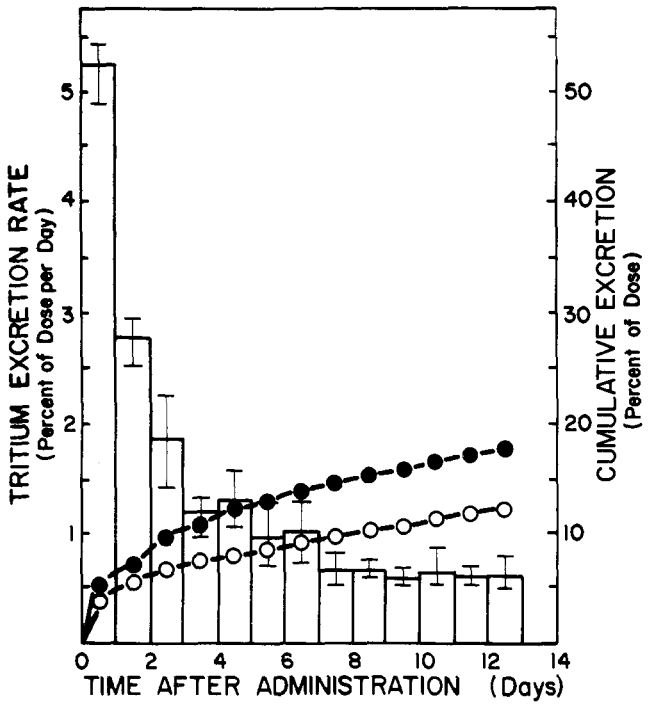


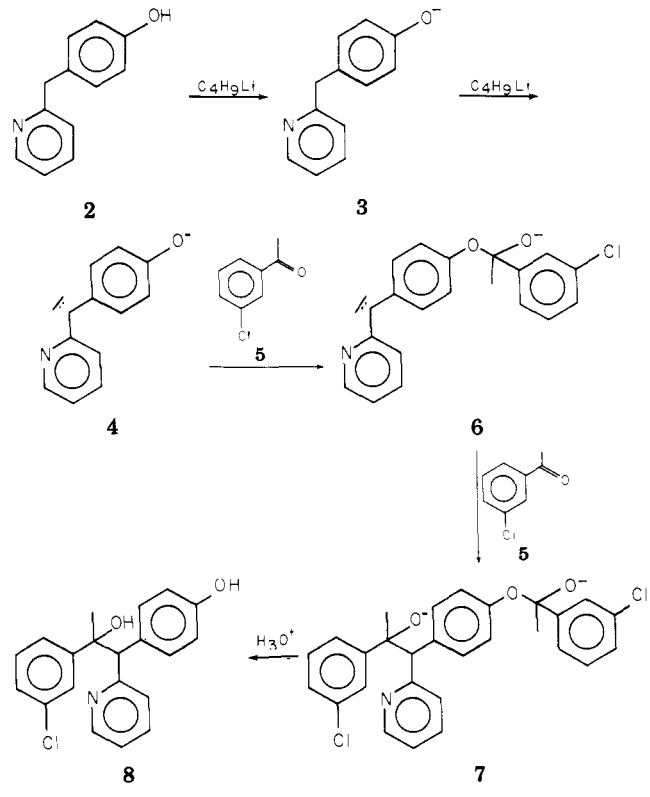
Figure 1. The average daily urine excretion for three rats is shown by histograms. Also, the cumulative average rate of excretion of radioactivity (•–•) is compared to the rate of excretion after loss of any volatile activity (○–○).

one exception in this regard was that a high percent of ^3H activity remained in the peritoneal cavity encapsulated in numerous white masses. This material was identified by TLC as compound 1 which had not been absorbed. Also included in Figure 1 are cumulative excretion rates. Curves were determined before and after volatile activity was removed by evaporation under N_2. The volatile activity was assumed to result from metabolically formed water. This conclusion is based upon the label stability of compound 1 under the experimental conditions employed and the lack of any TLC-detectable activity in the distillate. As shown in Figure 1 there was loss of volatile activity which is consistent with aromatic hydroxylation as an important mode of biotransformation.

Two rats were administered compound 1 (300 mg/kg, 15.6 μCi) ip for isolation and identification of metabolites. Balance studies for these rats were similar to those at the lower dosage level. Direct TLC examination of the urine of these two rats in system I clearly showed the absence of nonmetabolized drug at R_f 0.56. TLC in system II showed the following R_f bands and percentage distribution of radioactivity: 0.10 (31%), 0.28 (7%), 0.40 (6%), and 0.60 (56%). Enzymatic hydrolysis with either a β-glucuronidase–arylsulfatase mixture or β-glucuronidase per se, followed by TLC in system II, showed the loss of the 0.10 band with a corresponding increase mainly in the 0.60 band. Similar results were also obtained when radioactive material from the 0.10 band was isolated and was enzymatically hydrolyzed. It was therefore assumed that this band is predominantly the glucuronide conjugate of the 0.60 band.

Isolation of the major metabolite was either by preparative TLC of untreated urine in system II or by TLC of the acidic metabolites extracted from the Et_2O-soluble portion of the urine. The major metabolite was extracted from TLC plates with Et_2O and was silylated with bis(trimethylsilyl)acetamide in the presence of triethylamine before gas chromatography–mass spectrometry. The resulting mass spectrum exhibited a parent ion and

Scheme I



fragmentation pattern consistent with the silyl ether of a hydroxylated 2-benzylpyridine such as compound 2. However, the original drug, compound 1, under similar gas chromatography–mass spectrometry conditions underwent cleavage with a parent ion and fragmentation pattern consistent with 2-benzylpyridine. Therefore, the possibility of the major metabolite being a hydroxylated derivative of compound 1, such as compound 8, was also considered.

The para-hydroxylated compounds 2 and 8 were synthesized as the most reasonable reference compounds for comparison with the major metabolite. While compound 2 has been previously prepared by Sperber et al.,[3] their procedure was not satisfactory in our hands. Also, the Taylor et al.[4] procedure for direct hydroxylation of aromatic compounds with thallic trifluoroacetate failed when attempted with 2-benzylpyridine. But an overall 25% yield of compound 2 was obtained by starting with 2-benzylpyridine and nitration to 2-(4-nitrobenzyl)pyridine by the method of Schofield.[5] The nitro compound was hydrogenated with palladium on carbon to give the corresponding amino compound which was converted to the diazonium salt and was hydrolyzed to hydroxy compound 2. The amino compound was employed without purification in the diazonium salt reaction, but it should be used soon after its isolation since even at 0° it discolors within 1 day and its melting point drops upon further storage. Use of amino compound not freshly prepared led to reduced yields of 2. In addition, no product was obtained if the reaction mixture was kept hot beyond 5 min or if the diazonium salt was added slowly to boiling water.

Synthesis of compound 8, the hydroxylated derivative of the original drug 1, was accomplished (Scheme I) by modification of the procedure used to prepare 1.[2] Initial studies of this reaction, based upon color changes involved in formation of the dianion, indicated the *n*-butyllithium was a more satisfactory base than phenyllithium, lithium diisopropylamide, or sodium hydride. The first equivalent of base yields anion 3, appearing as a light yellow solution,

while the additional base reacts with a methylene hydrogen to form the dark dianion **4**. When only 1 equiv of 3-chloroacetophenone (**5**) was added at this point, followed by neutralization with dilute acid, work-up led only to recovery of starting materials. Also, if the ketone was added slowly to the dianion **4**, the red color did not lighten substantially until the second equivalent was being added, indicating that the oxygen anion reacts faster than the carbanion. It was therefore postulated that the addition of 1 equiv of 3-chloroacetophenone forms the hemiketal **6**. Addition of 2 equiv of the ketone allowed reaction with each anionic center to form **7**. The reaction was completed by acidic hydrolysis of the hemiketal to yield compound **8**.

Work-up led to a sticky oil which contained, in addition to considerable 3-chloroacetophenone (**5**) and a small amount of 2-(4-hydroxybenzyl)pyridine (**2**), significant amounts of racemates of the desired product as determined by TLC and NMR. Repeated extraction of the oil with hexane removed most of the acetophenone leaving a tan powder. Isolation of the two racemates was first accomplished by preparative TLC and silica gel column chromatography. In a later run a high melting racemate (mp 128–130.5°) was obtained in 20% yield from the crude solid by crystallization from chloroform–petroleum ether. The low-melting racemate was still obtained only by a column chromatographic separation followed by recrystallization from methylene chloride–petroleum ether (20% yield, mp 93–95°). The low-melting racemate does not have a very distinct melting point, as it softens at 75°. The racemate also retains solvents tenaciously. It was necessary to dry the analytical sample extensively before a satisfactory C, H, N analysis was obtained.

The choice of which racemate of hydroxy compound **8** is analogous stereochemically to the racemate of compound **1** used in the metabolic study is based upon NMR spectra. The resonances of the benzylic –CH– proton and the methyl group protons are of primary importance. For the low-melting racemate (**8a**), they appear at δ 4.36 (s, 1 H), and 1.22 (s, 3 H), and for the high-melting isomer (**8b**) they appear at δ 4.11 and 1.57. The aromatic region was not useful for racemate identification. The –CH– and CH_3– resonances of racemates **8a** and **8b** corresponded to those of the unhydroxylated drug (**1a** and **1b**). Since racemate **1a** (mp 134–136°) with resonance at δ 4.40 and 1.30 was the more hypocholesteremic of the two racemates used in the metabolic study, the hydroxy racemate **8a** with corresponding resonance (δ 4.36 and 1.22) should be the racemate derived both chemically and metabolically from **1a** and therefore of the same stereochemistry.

Product analysis, extent of reaction, and sample purity were easily determined by NMR. From such spectra of the crude products, more of the δ 4.11–1.57 isomer (**8b**) is formed than of racemate **8a**. However, equal amounts of each racemate were usually isolated. Composition of the crude product was also determined by TLC in system III in which the order of mobility is **5** > **8b** > **8a** > **2**. The compounds were eluted in the same order with silica gel column chromatographic separation. In a large-scale separation requiring 4 days of continuous elution, no racemate **8b** was obtained. It apparently decomposed on the silica gel while the **8a** isomer was unaffected.

TLC comparison (systems I–III) of samples of reference compounds with rat urine samples indicated that one of the minor metabolites cochromatographed with hydroxybenzylpyridine (**2**) and that the major metabolites cochromatographed with hydroxy compound **8a**. The presence of these two metabolites was confirmed by reverse isotope dilution. After enzymatic hydrolysis hydroxybenzylpyridine (**2**) and hydroxy compound **8a** accounted for 1 and 70%, respectively, of the total urine radioactivity.

Table I. Hypocholesteremic Activity of Compounds in Rats[a]

Compd	No. of animals	Serum cholesterol,[b] mg/100 ml	Depression, %	p[c]
Control	16	71.3 ± 8.6		
1	8	36.0 ± 5.9	49.5	>0.001
2	3	66.0 ± 2.0	7.4	n.s
8a	8	41.0 ± 5.6	43.5	>0.001

[a] Dose, 25 mg/kg in 200-g rats for 7 days. [b] See ref 7 for method of determination. Recorded as the mean ± standard deviation. [c] Student's t test.

Both metabolites were evaluated for hypocholesteremic effects in comparison with compound **1**. In all cases, male rats (200 g) were dosed orally with 25 mg of compound/kg/day for 7 days and were compared with control rats dosed in the same manner with only the vehicle. At the end of the 7 days, serum was assayed for cholesterol.[7] Results are summarized in Table I. Hydroxy compound **8a** demonstrated activity in rats similar to that of the original drug **1** while no hypocholestermic activity was established for hydroxybenzylpyridine (**2**).

Experimental Section

A Pye 014 chromatograph was used with a AEI MS double beam mass spectrometer for GC–MS measurements of metabolites. Mass spectra for the synthesized compounds were obtained with a GC–MS LKB 9000 S. Melting points were determined with a Mel-Temp apparatus; they are uncorrected. NMR spectra were recorded by means of a Varian A-60A spectrometer using $CDCl_3$ as the solvent and Me_4Si as an internal standard. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, Ind.

The following TLC systems were employed with either E. Merck silica gel 60 F_{254} analytical or preparative plates: I, hexane–chloroform–acetic acid (6:3:1); II, benzene–methanol (8:2); III, benzene–ethyl acetate (9:1) and IV, ethyl acetate–acetic acid (6:1).

Liquid scintillation counting was with a Beckman Model LS 200 spectrometer employing a fluid of 8 g of PPO and 0.4 g of POPOP in 1 l. of toluene. Aqueous samples required the addition of 20 ml of Scintisol GP per liter of the toluene mixture. Feces and tissue samples were oxidized with a Packard Model 300 tritium oxidizer and were counted in fluid of 40 parts of Triton X-100 and 60 parts of a solution of 8 g of PPO and 0.7 g of POPOP in 1 l. of toluene and were counted by means of a Packard Tri-Carb Model 3320 spectrometer. Standard compounds were detected by fluorescent quenching and radioactivity was quantitated by liquid scintillation counting of 0.5-cm strips of silica gel scraped from the plates.

Metabolic Balance Studies. Three male Sprague–Dawley rats (250 ± 10 g) were housed in separate 20 × 11.5 cm stainless steel metabolic cages with Mouse/Rat Diet (Teklad Inc.) and water supplied ad libitum. Rats were administered 1 ml of an aqueous–Tween 80 (0.26%) suspension of tritium labeled[2] compound 1 (40 mg/kg, 1.71 μCi) ip. Urine and feces were collected 11 h after administration and then daily for 13 days. Feces were collected daily, air-dried, powdered, and oxidized before counting. Animals were sacrificed 14 days after administration of compound 1, and selected tissues were measured for activity after oxidation. The volatile component of urine was estimated by comparing the counts of 0.1-ml aliquots before and after drying under N_2 in a scintillation vial. Dried samples were redried in the same manner after the addition of 1 ml of MeOH. For selected samples volatile material carried over by the N_2 was condensed in a dry ice–acetone trap. This condensate was counted and also was examined by TLC for the presence of 2-benzylpyridine.

Metabolite Identification. Two rats (250 g) were administered compound 1 (300 mg/kg, 15.6 μCi) and monitored in a manner similar to the previous balance study. Urine samples were

compared in TLC system II, before and after hydrolysis at pH 5.8 at 37° for 72 h with either β-glucuronidase–arylsulfatase (*Helix pomatia*, 3500 Fishman units per milliliter of urine, Sigma Chemical) or β-glucuronidase (bovine liver, 10000 units per milliliter of urine, Sigma Chemical). The major metabolite and its conjugate were isolated from the MeOH-soluble portion of the dried urine by preparative TLC with system II. For the isolation of larger quantities of metabolites and their conjugates, pooled urine was adjusted to pH 6 and was extracted for 72 h with Et_2O. The NaOH-soluble portion of this Et_2O extract was the only Et_2O fraction of high activity. A crude free metabolite fraction was isolated from it by adjustment of pH to 6 followed by Et_2O extraction. A corresponding crude conjugated metabolite fraction was obtained from the aqueous urine layer after Et_2O extraction by freeze drying and MeOH extraction of the residue. The crude conjugate was hydrolyzed with β-glucuronidase–arylsulfatase as previously described, and it was isolated by Et_2O extraction. The free metabolites and those isolated from their conjugates were further purified by preparative TLC in system II.

The front-running radioactive bands in these separations (R_f 0.6) were extracted with Et_2O and silylated with bis(trimethylsilyl)acetamide (10 ml); triethylamine (10 ml) was added to an Et_2O solution of metabolite (30 μl, ca. 40 μg). Portions (5 ul) were separated by GLC on 3% SE-30 Chromosorb G column and fractions collected in scintillation fluid for measurement of radioactivity. In a subsequent run the only fraction corresponding to an intense peak which had radioactivity was introduced directly into the mass spectrometer. The following fragmentation pattern resulted at 70 eV: 257, parent ion (P); 242, P – CH_3; 179, P – C_5H_4N; 168, P – $OSi(CH_3)_3$; 92, P – $C_6H_5OSi(CH_3)_3$; 73, $Si(CH_3)_3$.

2-(4-Aminobenzyl)pyridine. To a solution of 2-(4-nitrobenzyl)pyridine (10.0 g, 0.0467 mol) dissolved in benzene (250 ml), 5% palladium on carbon (0.50 g) was added. The solution was hydrogenated in a Parr apparatus at 20 psi until hydrogen uptake ceased. The light yellow solution was filtered and the filtrate was taken to dryness leaving a light yellow oil which crystallized upon standing as nearly colorless crystals (8.32 g, 97%): mp 60–62° (lit.[6] mp 58–60°).

2-(4-Hydroxybenzyl)pyridine (2). 2-(4-Aminobenzyl)-pyridine (8.30 g, 0.045 mol) was dissolved in a 50% aqueous H_2SO_4 solution (48 ml). While maintaining the solution at less than 5°, $NaNO_2$ (3.76 g, 0.045 mol) in H_2O (16 ml) was added dropwise with stirring during 5 min. The resulting light brown mixture was added during 5 min to rapidly stirred boiling H_2O (400 ml). After a further 5 min, N_2 evolution had ceased. The solution was poured over ice and a small amount of urea added. The cold solution was neutralized to pH 7 with cold concentrated NH_4OH. The brown, gummy precipitate was extracted with CH_2Cl_2 (5 × 100 ml); the extracts were combined, dried (K_2CO_3), and taken to dryness leaving a dark brown sticky solid (6.00 g) which was sublimed at 140° (0.2 Torr). The yellow sublimate was crystallized as fine needles (4.60 g, 55%): mp 132–133° (lit.[3] mp 129.5–130.5°); mass spectrum (70 eV) *m/e* (rel intensity) 185 (48), 184 (100), 183 (10), 167 (7), 107 (15), 77 (8).

(*RS*)-1-(3-Chlorophenyl)-2-(4-hydroxyphenyl)-1-methyl-2-pyridineethanol (8). To a solution of **2** (6.00 g, 0.0324 mol) in dioxane (200 ml, freshly distilled from sodium), cooled in an ice bath under a dry N_2 atmosphere, a solution of *n*-butyllithium in hexane (32.4 ml of a 2.0 M solution) was added during 15 min. The resulting red dianion mixture was stirred at 0° for 0.5 h and 3-chloroacetophenone (10.02 g, 0.648 mol) in dioxane (50 ml) was added during 1 min. The resulting yellow solution was stirred at room temperature for 0.5 h. The reaction mixture was recooled to 0° and was poured into a solution of 38% HCl (5.4 ml) in H_2O (50 ml). The resulting mixture was poured into a mixture of Et_2O (200 ml) and H_2O (50 ml). The organic layer was drawn off and the aqueous layer (pH 7) was extracted with Et_2O (2 × 100 ml). The combined ether layers were extracted with H_2O (2 × 100 ml), were dried (K_2CO_3), and were taken to dryness leaving a red-brown oil which was triturated several times with 50-ml portions of hexane until a tacky solid remained. Crystallization from $CHCl_3$–petroleum ether gave a white crystalline solid (2.62 g) which was recrystallized as a fine, white crystalline racemate **8b** (2.18 g, 20%): mp 128–130.5°; NMR ($CDCl_3$) δ 7.81–6.32 (m, 14 H), 4.11 (s, 1 H), 1.57 (s, 3 H). Anal. ($C_{20}H_{18}ClNO_2$) C, H, N.

The mother liquor from the first $CHCl_3$–petroleum ether crystallization of **8b** was taken to dryness and red-brown gum eluted through a silica gel column (Woehm, 30 × 300 mm) with benzene–ethyl acetate (9:1) as the solvent. The first fractions contained 3-chloroacetophenone and a second set contained more of the above racemate **8b**. The third set of fractions containing the other *RS* pair was combined and taken to dryness affording a white solid **8a** (3.91 g) which was crystallized from CH_2Cl_2–petroleum ether as white needles (2.15 g, 20%): mp 93–95°; NMR ($CDCl_3$) δ 7.58–6.58 (m, 14 H), 4.36 (s, 1 H), 1.22 (s, 3 H); mass spectrum (70 eV) *m/e* (rel intensity) 185 (71), 184 (100), 183 (14), 154 (30), 139 (60), 141 (20), 111 (32), 107 (17), 86 (23), 84 (37), 97 (13), 77 (14), 75 (14). Anal. ($C_{20}H_{18}ClNO_2$) C, H, N.

Compound **8a** was silylated as previously described for the metabolite and the product separated by GLC on 1% OV-1 on Chromosorb G prior to direct introduction into the mass spectrometer: mass spectrum (70 eV) *m/e* (rel intensity) 257 (34), 256 (35), 242 (19), 179 (8), 168 (7), 120.5 (25), 121 (7), 92 (30), 75 (30), 74 (10), 73 (100).

Comparison of Metabolites with Reference Compounds. Compounds **1**, **2**, **5**, **8a**, and 2-benzylpyridine were co-spotted with samples of urine before and after hydrolysis in systems I–III. These chromatograms were consistent with **2** in trace amounts and with **8a** as the major free metabolite as well as the major aglycon of the conjugated metabolites. There was no indication of the presence of the other reference compounds. Therefore, reverse isotope dilution studies were conducted to establish the presence of total free and conjugated compounds **2** and **8a**. Compound **2** (0.5 g) was added to 5 ml of urine and the recovered compound **2** was recrystallized to constant specific activity alternating between MeOH and benzene. For compound **8a**, 0.5 g of compound **8a** was added to 4 ml of urine and the recovered compound **8a** was recrystallized three times from acetone–hexane followed by two times from benzene–MeOH.

Measurement of Cholesterol. Male Sprague–Dawley rats of approximately 200 g were supplied Mouse/Rat Diet (Teklad Inc.) and water ad libitum before and during the experiment. Rats were administered aqueous suspensions of compounds **1**, **2**, and **8a** (25 mg/kg) by oral intubation daily for 7 days. Blood samples were taken by closed chest cardiac puncture 24 h after the last administration of drug and were analyzed for cholesterol by the method of Block et al.[7] Control values were obtained in the same manner but with the administration of H_2O in place of aqueous drug suspensions.

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References and Notes

(1) J. H. Burckhalter, W. D. Dixon, M. L. Black, R. D. Westland, L. M. Werbel, H. A. DeWald, J. R. Dice, G. Rodney, and D. H. Kaump, *J. Med. Chem.*, **10**, 565 (1967).
(2) D. W. Hansen, Jr., E. Van Den Eeckhout, J. C. Drach, J. H. Burckhalter, and J. E. Sinsheimer, *J. Labelled Compd.*, **10**, 213 (1974).
(3) N. Sperber, D. Papa, E. Schwenk, and M. Sherlock, *J. Am. Chem. Soc.*, **73**, 3856 (1951).
(4) E. C. Taylor, H. W. Altland, R. H. Danforth, G. McGillivray, and A. McKillop, *J. Am. Chem. Soc.*, **92**, 3520 (1970).
(5) K. Schofield, *J. Chem. Soc.*, 2408 (1949).
(6) M. Hamana and K. Funakoshi, *Yakugaku Zasshi*, **82**, 523 (1962).
(7) W. D. Block, K. J. Jarrett, Jr., and J. A. Levine, *Clin. Chem.*, **12**, 681 (1966).