and the mixture was stirred for 20 min in N_2 atmosphere. An O_2 stream was then introduced to this mixture for 20 min under ice cooling. The mixture was poured onto ice–AcOH and extracted with $CHCl_3$. The residue (56 mg) of the extract was refluxed with DDQ (40 mg) in dioxane (6 ml) for 30 min. Chromatographic purification (silica gel, 8 g) gave **22** (13 mg, MeOH, mp 275–276° dec): MS m/e 410 (M^+). Anal. ($C_{25}H_{18}N_2O_4$) C, H, N.

(12) *dl*-Desethylphenacylcamptothecin (23) from 17. **17** was treated analogously as in experiments 9 and 10: **17** (50 mg), K_2CO_3 (64 mg), phenacyl bromide (46 mg) in acetone (6 ml), concentrated HCl (0.2 ml), $Cu(OAc)_2 \cdot H_2O$ (4 mg), $(Et)_3N$ (21 mg) in MeOH (6 ml) with an O_2 stream (20 min), and DDQ (64 mg) in dioxane (8 ml). Chromatographic purification (silica gel, 12 g) gave **23** (7 mg, $CHCl_3$–MeOH, mp 267–268° dec): MS m/e 438 (M^+). Anal. ($C_{26}H_{18}N_2O_5$) C, H, N.

(13) 25 from 17 via 24. **17** (50 mg) was treated analogously as in the above experiment with K_2CO_3 (64 mg) and ethyl bromoacetate (38 mg) in acetone (6 ml), followed by concentrated HCl (0.2 ml). The resulting residue (42 mg) was kept in saturated HCl gas–EtOH (4 ml) for 30 min. EtOH was evaporated and the residue was treated with ice–AcONa and extracted with $CHCl_3$. The residue (38 mg) of the extract was treated analogously as in the above experiment: $Cu(OAc)_2 \cdot H_2O$ (4 mg), $(Et)_3N$ (20 mg) in EtOH (5 ml) with O_2 stream (20 min), and DDQ (38 mg) in dioxane (5 ml). Chromatographic purification (silica gel, 6 g) gave **25** (7 mg, MeOH, mp 234–235° dec): MS m/e 480 (M^+). Anal. ($C_{26}H_{28}N_2O_7$) C, H, N.

Attempted Synthesis of 29 from 17. A solution of **17** (100 mg, 0.23 mmol) in pyridine (2 ml) and DMF (1 ml) containing acrolein (38 mg, 0.69 mmol) was kept for 20 h in the dark. After concentration of the solvent followed by codistillation with toluene, the residue was chromatographed [silica gel, 2 g; solvent system $CHCl_3$–CH_3OH (99:1); effluent 150 ml] to give a mixture of epimers of crude **26** (113 mg): NMR ($CDCl_3$) 1.2 (9 H, s, *t*-Bu), 8.5 (1 H, s, NCHO), 9.7 ppm (1 H, s, CHO); ir ($CHCl_3$) 1760, 1740, 1630 cm^{-1}. A mixture of crude **26** (113 mg, 0.23 mmol) in absolute benzene (5 ml) containing formic acid (25 mg, 0.55 mmol) and $(Et)_2NH$ (20 mg, 0.28 mmol) was refluxed for 2 h under stirring. After being cooled, the mixture was extracted with $CHCl_3$–MeOH (3:1). The residue of the extract was chromatographed (silica gel, 1 g, solvent system $CHCl_3$ containing 1–5% MeOH) to give a mixture of epimers of crude **27** (94 mg): ir ($CHCl_3$) 1760, 1740, 1640 cm^{-1}; NMR 0.9–1.2 (15 H, *t*-Bu + $2CH_3$), 8.6 ppm (1 H, s, NCHO). A solution of crude **27** (94 mg) in concentrated HCl (0.2 ml) was allowed to stand for 16 h at room temperature. The solution was then treated with AcONa and extracted with $CHCl_3$–MeOH (3:1). The residue (78 mg) of the extract was dissolved in AcOH (2 ml) and the solution was treated with an O_2 stream for 20 min under refluxing. The solution was then reduced and the residue was treated with ice–Na_2CO_3 and then extracted with $CHCl_3$–MeOH (3:1). The residue (72 mg) of the extract was chromatographed (silica gel, 1 g; solvent system $CHCl_3$ containing 3–10% MeOH) to give a residue (27 mg, tentatively **28**). A suspension of this residue (27 mg), $Cu(OAc)_2 \cdot H_2O$ (2 mg), and $(Et)_3N$ (5 mg) in MeOH (2 ml) was treated with an O_2 stream for 40 min. CH_3OH was removed at room temperature and the residue was treated with ice–AcOH and then extracted with $CHCl_3$. The residue of the extract was purified by TLC [solvent system $CHCl_3$–MeOH (3:1)]. The main fraction (5 mg) was recrystallized to give the product (2 mg, mp 235–237° dec): MS m/e 433 (M^+); ir ($CHCl_3$) 1750, 1640 cm^{-1}. Anal. ($C_{25}H_{27}N_3O_6$) C, H, N.

Pharmacology. Material and Method. Animals and Tumor. BDF_1 mice of 5 weeks of age were used, four males and four females for test groups, five males and five females for control groups. Mice were inoculated intraperitoneally with 5×10^5 cells of L1210. Test compound was injected intraperitoneally once on the day or five times for 5 days following tumor inoculation. Antitumor activity was evaluated by the increase in life span over controls.

Test Compounds. Chemical structures are listed in Table I. Since the compounds except **13** sodium salt are insoluble, they were suspended in the suspending vehicle[5] before using.

References and Notes

(1) A. G. Schultz, *Chem. Rev.*, **73**, 389 (1973).
(2) (a) T. Sugasawa, T. Toyoda, and K. Sasakura, *Tetrahedron Lett.*, 5109 (1972); (b) *Chem. Pharm. Bull.*, **22**, 771 (1974); (c) *ibid.*, **22**, 763 (1974).
(3) W. Bartok and H. Pobiner, *J. Org. Chem.*, **30**, 274 (1965).
(4) M. E. Wall, 4th International Symposium on the Biochemistry and Physiology of Alkaloids, Hall, DDR, June 25–28, 1969; *Abh. Dtsch. Akad. Wiss. Berlin*, 46 (1971).
(5) NaCl, 0.9%; Polysorbate 80, 0.4%; CMC, 0.5%; benzyl alcohol, 0.9% in 100 ml of water.

Solid-Phase Synthesis of Drug Glucuronides by Immobilized Glucuronosyltransferase

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Rabbit liver glucuronosyltransferase immobilized on beaded agarose has been used to synthesize glucuronic acid conjugates of meprobamate, diethylstilbestrol, bilirubin, borneol, benzoic acid, and *p*-nitrothiophenol. The immobilized enzyme exhibited a high degree of specificity for UDPGA as cofactor when *p*-nitrophenol is used as substrate. Other cofactors tested were less effective, all producing less than 10% conjugation relative to UDPGA. The effects on agarose-bound enzyme activity of a variety of cosolvents and emulsifiers have been studied. Ethanol, dimethyl sulfoxide, propylene glycol, and bovine serum albumin are among the cosolvents and emulsifiers which can be used within limited concentration ranges to solubilize lipophilic substrates for conjugation. Concentrations of calcium and magnesium cations between 1.5 and 10.0 mM were found to enhance glucuronosyltransferase activity of the immobilized enzyme.

A nearly universal theme in the metabolism of low-molecular-weight drugs and pesticides is their conjugation to glucuronic and sulfuric acids. The resulting conjugates are water soluble, ionized, and usually more readily cleared from the body than the aglycon. The critical role that glucuronide and sulfate conjugates play in controlling the availability of steroid hormones, catecholamines, heme catabolites, and other endogenous compounds is also currently being explored.

Identification of conjugates in most contemporary studies is usually inductive, not direct, based on confirmation of the presence of aglycon after treatment with β-glucuronidase, sulfatases, or acid hydrolysis.[1] In the last several years a number of reports demonstrate that glu-

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Table I. Glucuronides Synthesized Using Immobilized Glucuronosyltransferase[a]

Aglycon	Yield, %	TLC, R_f
p-Nitrophenol	65	0.63
Borneol	54	0.69
Diethylstilbestrol	26	0.65
Meprobamate	31	0.12, 0.53
Benzoic acid	5	0.58
Bilirubin	7	0.23
Nitrothiophenol	2	0.57

[a] Uniform conditions were used (see Experimental Section) rather than conditions optimized for each substrate.

curonides or various derivatives may be characterized by several chromatographic techniques and by mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance.[2-8] Thus the techniques exist for direct identification of intact glucuronides obtained in metabolic or biochemical studies. One major impediment to the application of these techniques is the difficulty in obtaining glucuronides of known structure for use as standards.

We have sought an enzyme-catalyzed synthetic method which would lead to cleaner products than those obtained in vivo or by microsomal incubation. At the same time it is intended to preserve the generality of the enzymatic approach to a wide range of substrates with a variety of conjugatable functional groups. To this end we recently reported the partial purification of UDPglucuronosyltransferase and its immobilization on beaded agarose.[9] Among the many questions which can be posed for this solid-phase approach to glucuronide synthesis, we address four in this paper.

A. Aglycon Variation. Glucuronyltransferases in vivo have been reported to accommodate a variety of substrates, including phenols, alcohols, amines, thiols, carbamates, and carboxylic acids.[1] This generality is highly desirable in the immobilized enzyme reagent as well; thus, a variety of substrates have been tested to see which can be conjugated.

In the experiment reported in Table I parallel incubations were run using portions of the same batch of immobilized enzyme reagent as well as the same temperature, time, and other conditions. Conjugates were extracted from the reaction mixture in butanol or ether, allowed to react with naphthoresorcinol,[1] and assayed spectrophotometrically. The yields in Table I were calculated against the amount of aglycon used in the reaction. The products were further characterized by their mobility on thin-layer chromatography and in some cases by gas chromatography and mass spectrometry.

The conditions used for the parallel incubations had been optimized for conjugation of *p*-nitrophenol. (At longer reaction times complete *p*-nitrophenol conjugation may be achieved.) Not surprisingly, yields of the other hydroxy-linked conjugates, borneol and diethylstilbestrol, are satisfactory as well. Diethylstilbestrol was solubilized for enzymatic conjugation by the addition of bovine serum albumin. We have found that the presence of albumin in the buffered solution inhibits conjugation of *p*-nitrophenol by agarose-bound glucuronosyltransferase (see discussion of cosolvents below). It is probably this inhibitory effect of albumin which lowers the yield of diethylstilbestrol glucuronide relative to borneol and *p*-nitrophenol glucuronides.

In contrast to amino glucuronides, which are notoriously unstable during chemical manipulation, the carbamate linkage in meprobamate glucuronide is relatively stable. For this reason meprobamate was chosen to test the ability of the immobilized transferase to conjugate nitrogen-containing functional groups. The yield reported (Table I) for meprobamate is calculated on the basis of an equimolar conjugate. Interestingly, two glucuronides were separated by thin-layer chromatography, presumably the mono- and the diglucuronides. Only the monoglucuronide has been detected as a human metabolite.

Benzoic acid was chosen as the simplest aromatic carboxylic acid aglycon. The yield reported is relatively poor, but enough material was obtained (ca. 4 mg) to characterize the compound by thin-layer and gas chromatography and by mass spectrometry. It should be pointed out that no attempt was made to find conditions which would provide optimal yields of carboxylate glucuronides.

Bilirubin was used to test the ability of the immobilized transferase system to conjugate an aliphatic carboxylic acid. It is unique among the compounds listed in Table I as a naturally encountered endogenous substrate for UDPglucuronosyltransferase. In this case the standard conditions for the incubation were altered to provide a Mg^{2+} containing buffer used by Strebel and Odell for optimal microsomal conjugation[10] of bilirubin. Conjugated bilirubin was assayed by the known spectrophotometric method.[10] The monoglucuronide but not the diglucuronide of bilirubin was detected. Bilirubin monoglucuronide was characterized by thin-layer chromatography against authentic material[11] and by conversion to two azo derivatives by treatment with sulfanilyldiazonium chloride. These latter products were also characterized by thin-layer chromatographic comparison with two azo derivatives obtained from authentic bilirubin monoglucuronide.[10]

The yield of *p*-nitrothiophenol glucuronide is the lowest in Table I and studies are underway to improve the yields of thio glucuronides. The experiment reported in Table I was not designed to reflect optimal yields of each substrate but to compare the extent to which different substrates are conjugated under standardized conditions.

The glucuronides of *p*-nitrophenol, borneol, and benzoic acid were converted to tris(trimethylsilyl)methyl esters and further characterized by gas chromatography–mass spectrometry. The *p*-nitrophenol glucuronide derivative eluted at 3.2 min from a 6-ft 0.5% Dexil column heated isothermally at 250°. On a 6-ft 3% Dexil column temperature programmed from 150° at 6°/min, the borneol glucuronide derivative was eluted at 21.5 min. When the same column was heated from 180° at 6°/min, tris(trimethylsilyl)benzoyl glucuronide methyl ester was eluted at 11.5 min. The sample of borneol used as substrate for conjugation was contaminated (approximately 15%) with isoborneol, and corresponding isomeric glucuronides were separated by gas chromatography, whose mass spectra were very similar. The minor component was eluted faster from the Dexil column, both in the aglycon sample and in the conjugate product mixture. Tetrakis(trimethylsilyl)diethylstilbestrol glucuronide methyl ester could be separated on 3% Dexil from unconjugated bis(trimethylsilyl)diethylstilbestrol and impurities introduced by the silylation procedure. It eluted above 300° over a period of several minutes.

Electron-impact mass spectra were obtained of the glucuronides as they eluted from the gas chromatograph. The spectrum of the *p*-nitrophenol glucuronide derivative has been described before.[2] The spectra of the tris(trimethylsilyl)methyl ester derivatives of the glucuronides of borneol and benzoic acid and also of the tetrakis(trimethylsilyl)methyl ester of diethylstilbestrol are presented in Figures 1–3. The spectrum of the borneol glucuronide derivative corresponds well to the general features of trimethylsilylated glucuronide spectra[2] and to the spec-

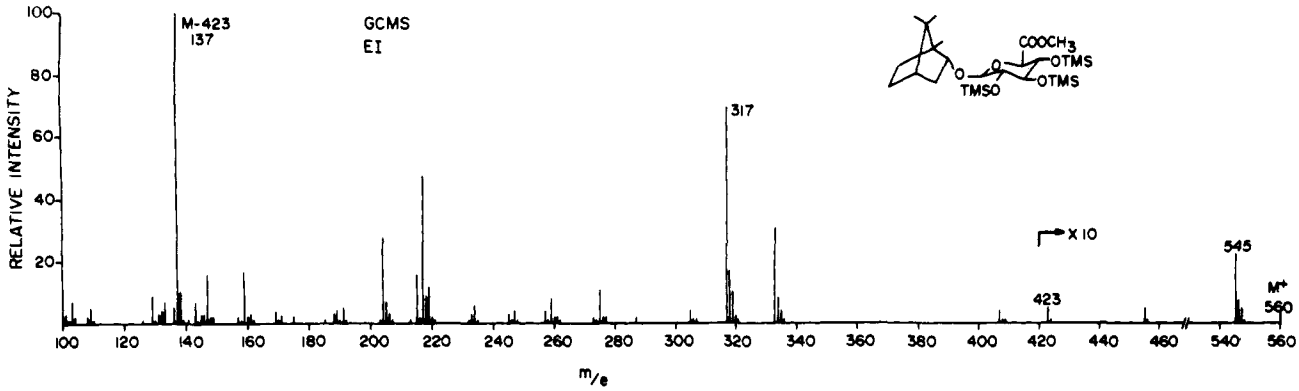


Figure 1. Electron-impact mass spectrum of tris(trimethylsilyl)borneol glucuronide methyl ester.

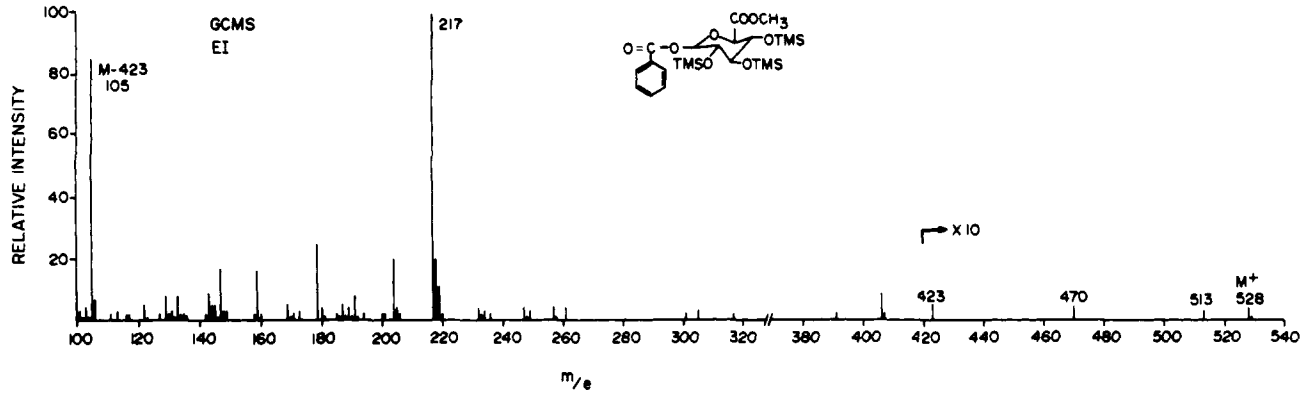


Figure 2. Electron-impact mass spectrum of tris(trimethylsilyl)benzoyl glucuronide methyl ester.

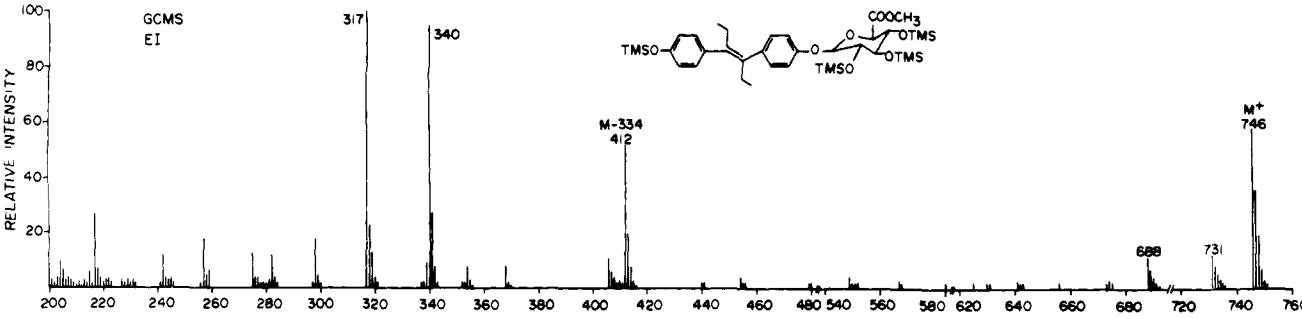
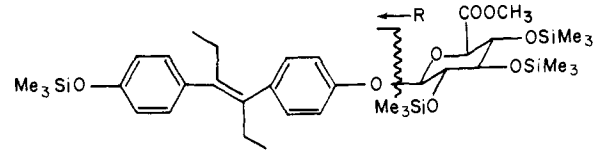


Figure 3. Electron-impact mass spectrum of tetrakis(trimethylsilyl)diethylstilbestrol monoglucuronide methyl ester.

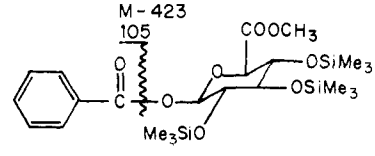
trum of borneol glucuronide isolated from urine of a patient poisoned with camphorated oil.[12] Most notably, the intensity ratio of the peaks M - 334/M - 423 is less than 1/6 (and is, in fact, 1/100) as is predicted for an aliphatic hydroxy-linked conjugate. In the spectrum of diethylstilbestrol glucuronide (Figure 3) the intensity ratio of the peaks M - 334/M - 423 is 26/1, appropriate for an aromatic hydroxy-linked glucuronide.[2] The molecular ion is quite abundant in this spectrum, which also exhibits the usual peaks associated with the silylated sugar moiety.[2] Spectra obtained both by gas chromatographic introduction of the sample and by introduction on the direct probe contain abundant M - 406 peaks at *m/e* 340. These ions probably are formed by cleavage in the acetal linkage accompanied by transfer of a hydrogen atom, analogous to the formation of M - 334 ions.

The spectrum of the derivative of benzoic acid glucuronide (Figure 2) contains a "molecular ion set"[2] of peaks corresponding to M^+, M - 15, and M - 58. Although an ion from the sugar moiety contributes the base peak at *m/e* 217, ions are also formed in abundance by cleavage at the carbonyl group (mass 105). Cleavage at the carbonyl group leads to loss of 423 mass units and the M - 334/M - 423 ratio for this spectrum is 1/83. Such facile cleavage of the



R = H, M - 406, *m/e* 340
R = Me_3Si, M - 334, *m/e* 412

ketal linkage at the carbonyl group is likely a general characteristic of the electron-impact induced fragmentation of glucuronides of aromatic acids.



B. Other Glycosyltransferase Activity. In a second study we have searched for other glycosyltransferases associated with the agarose-bound crude glucuronosyltransferase. Uridinediphosphoglucuronic acid (UDPGA) in the incubation medium was replaced with a variety of

Table II. Conjugation of Alternate Cofactors

Cofactor	% p-nitrophenol conjugated
Uridinediphosphoglucuronic acid	42.0
Uridinediphosphogalacturonic acid	1.5
Uridinediphosphoglucose	3.3
Uridinediphosphogalactose	3.2
Uridinediphospho-*N*-acetylglucosamine	4.5
Uridine triphosphate	2.5

Table III. Enzyme Inhibition by the Cosolvents

Cosolvent	Concn for 50% inhibn, %
Ethanol[b]	17
Dimethyl sulfoxide	13
Propylene glycol	11
Glycerol	6
Dimethylformamide	6
Dioxane[b]	<2
Tween-30	<0.5
Bovine serum albumin	1.2[a]
Luberol-PX	0.5[a]

[a] The percent concentration is expressed in w/v (g/100 ml). All other cases are v/v. [b] See ref 9.

potential cofactors as shown in Table II. Assaying *p*-nitrophenol as the substrate, we find that less than 5% is conjugated to any of the other glycosides under conditions where 42% is conjugated to glucuronic acid. *N*-Acetylglucosamine is the next most effective sugar to be conjugated to *p*-nitrophenol. Thus the rabbit liver transferase immobilized by our procedure is relatively specific for uridinediphosphoglucuronic acid, when *p*-nitrophenol is used as substrate.

C. Cosolvents. Many drugs and pesticides which are conjugated in vivo have little or no solubility in the aqueous medium required by the enzymatic method described here. If glucuronides are to be synthesized from these hydrophobic compounds, some cosolvent or emulsifier must be used to render them accessible to the enzyme. At the same time any cosolvent or emulsifier used must not destroy the transferase activity of the immobilized enzyme. Thus a study has been carried out on the effects of various cosolvents, emulsifiers, and detergents on the enzyme activity.

Table III presents the concentrations of these cosolvents at which transferase activity has been reduced by 50%. Thus 1% (w/v) of albumin may be added, or 13% (v/v) of dimethyl sulfoxide may be added, without inhibiting the immobilized transferase activity by more than one-half. Ethanol is used in almost all the incubations, including the *p*-nitrophenol assay[9] where its concentration in the final incubation solution is 0.5%. Slightly more (0.7%) was used in the conjugation of borneol. Bovine serum albumin (2.5% w/v) was used to solubilize diethylstilbestrol for conjugation. The inhibition of glucuronyl transferase activity by albumin has been reported for microsomal incubations as well, and the explanation has been advanced that albumin-bound aglycons are not substrates for the enzyme.[13]

D. Cation Requirements. A recent study by Howland et al.[14] of the effects of magnesium and calcium cations on the transferase activity of microsomal incubations suggested that yields might be improved with the immobilized system at the correct cation concentration. Indeed, low concentrations of magnesium chloride or calcium chloride were found to enhance the rate of conjugation favorably (Figures 4 and 5). The optimum

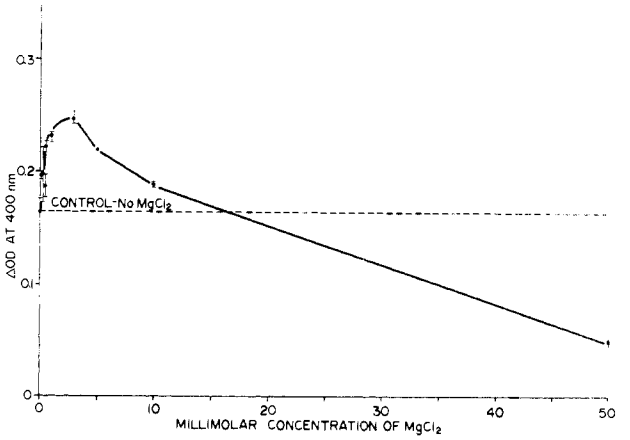


Figure 4. Effect of $MgCl_2$ on the activity of agarose-bound glucuronosyltransferase, assaying with *p*-nitrophenol as substrate at 37° for 90 min. High, low, and average of three measurements are shown for each point.

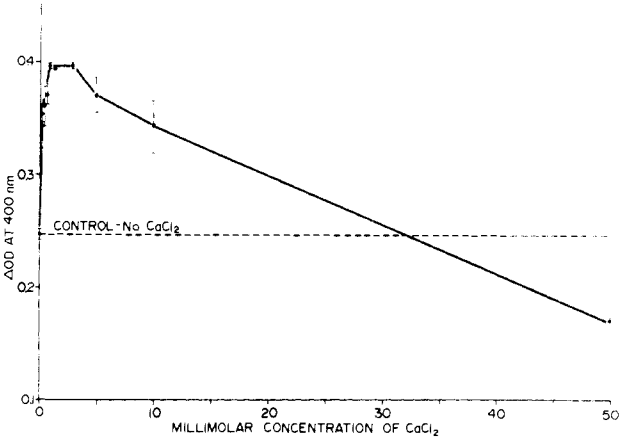


Figure 5. Effect of $CaCl_2$ on the activity of agarose-bound glucuronosyltransferase, assaying with *p*-nitrophenol as substrate at 37° for 90 min. High, low, and average of three measurements are shown for each point.

concentration of Mg^{2+} in the enzymatic incubation medium with *p*-nitrophenol as substrate occurs around 3.0 mM and that of Ca^{2+} plateaus in the range 0.8–3.0 mM. Figures 4 and 5 also indicate that inhibition occurs at higher concentrations. Fifty percent inhibition occurs at 37.5 mM magnesium chloride and at 71.5 mM calcium chloride. Additive effects of Ca^{2+} and Mg^{2+} were not studied. Although neither calcium chloride nor magnesium chloride has been added to the other incubations discussed in this paper (e.g., Tables I–III, excluding bilirubin), it seems likely that incorporation of such metal ions will increase yields in many cases.

Experimental Section

Agarose-bound glucuronosyltransferase was prepared according to the procedure published earlier,[9] except that activation of the agarose beads was modified to follow a recent procedure of March et al.[15]

Assays for glucuronosyltransferase activity were based on the spectrophotometric determination of *p*-nitrophenol described earlier.[9] In this work, incubations were carried out in 3-ml tubes at 37° through 90 min. The incubation solution (200 μl) was withdrawn and quenched with 800 μl of glycine buffer, pH 10.4. In contrast to our earlier studies only UDPGA was omitted in controls.

One activity unit is defined as that amount of agarose-bound enzyme which will conjugate 1 μmol of *p*-nitrophenol in 60 min at pH 8.0, 37 °C.

Glucuronide syntheses were carried out following the procedure reported earlier,[9] with *p*-nitrophenol, borneol, di-

ethylstilbestrol, benzoic acid, meprobamate, and *p*-nitrothiophenol. Equimolar amounts (0.2 mmol) of each aglycon and UDPGA were incubated (in parallel) with 17 activity units from the same batch of agarose-bound enzyme for 17 h at 37°, on a rotating shaker. The gel in each incubation mixture was filtered and washed under suction three times with 15 ml of 0.05 M Tris buffer, pH 8.0. The combined filtrates were adjusted to pH 8 (borneol, meprobamate, diethylstilbestrol) or pH 3.5 (benzoic acid, *p*-nitrophenol, *p*-nitrothiophenol) and extracted repeatedly with ether until no more free drug could be detected in the ether wash by thin-layer chromatography.

The aqueous phase from the borneol reaction was subsequently adjusted to pH 3.5 and extracted twice with ether. An aliquot was removed from the combined ether solutions for spectrophotometric quantitation of glucuronide formation. The rest was evaporated to dryness and the glucuronide characterized by thin-layer chromatography and derivatized for gas chromatograph–mass spectrometric analysis.

Aqueous solutions of the other five conjugates were adjusted to or maintained at pH 3.5 and extracted three times with equal volumes of butanol. Because the butanol extracts still contained trace amounts of Tris·HCl, the residue obtained after evaporation of butanol (under reduced pressure) was triturated with 5 ml of butanol to preferentially dissolve the drug glucuronide. Evaporation and trituration two additional times afforded a Tris·HCl free preparation of the drug glucuronide. An aliquot of the final butanol solution was removed for spectrophotometric quantitation of the conjugates. The rest was evaporated and the glucuronide was characterized by thin-layer chromatography and derivatized for characterization by gas chromatography–mass spectrometry.

Bilirubin (7.5 mg) was solubilized in 0.3 ml of 0.25 M sodium hydroxide. This solution was added to a solution of 625 mg of bovine serum albumin in 10 ml of 0.1 M Tris buffer, pH 7.6. The total volume was adjusted to 25 ml with 0.1 M Tris buffer to provide a bilirubin concentration of 30 mg %. This solution (4 ml) was incubated with 30 ml of agarose-bound enzyme solution, 21 mg of UDPGA in 1.0 ml of distilled water, 2.5 ml of a 50 mM solution of magnesium chloride in 1 M Tris buffer, pH 7.6, and 2 ml of a 0.15 M aqueous solution of potassium chloride.[10] The incubation proceeded through 90 min at 37°. The solution was filtered and conjugated material was extracted following the procedure of Streble and Odell.[10] An aliquot was taken for spectrophotometric quantitation of the yield, following the published procedure. A second portion of the conjugated material was characterized by thin-layer chromatography.[11] A third portion was treated with sulfanilyldiazonium chloride[10] and the two products formed were characterized by thin-layer chromatography against standard materials.

Quantitation of glucuronides was carried out by spectrophotometric measurements of the colored product formed on reaction with acidic naphthoresorcinol.[1,9]

Thin-layer chromatography of all the glucuronides except bilirubin glucuronide was done on small silica GF plates (Analtech, Inc.) using butanol–acetic acid–water, 35:3:10.[16] Bilirubin glucuronide was chromatographed on silica gel 60 F-254 (E.M. Laboratories).

Gas chromatography–mass spectrometry was carried out on a Du Pont 491 instrument using 6-ft glass columns packed with 3% Dexsil on Supelcoport, programmed from 150° or from 180° at 6°/min. The injection port was maintained at 270°, the jet separator at 250°, and the ionization source of the mass spectrometer at 200°. Mass spectra were also measured on a CEC 21-110 B mass spectrometer, introducing the sample via the direct probe. Glucuronides were derivatized for mass spectrometric analysis by treatment with diazomethane (generated from Aldrich Diazald) and then with bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane.[2]

Other glycosyltransferase activities were evaluated by substituting uridinediphosphogalacturonic acid, uridinediphosphoglucose, uridinediphosphogalactose, and uridinediphospho-*N*-acetylglucosamine for UDPGA in the standard assay.

Inhibition of transferase activity by cosolvents and emulsifiers was assayed using *p*-nitrophenol as substrate. The material being tested was added to the incubation mixture[9] in the Tris buffer. Inhibition was studied at five or six concentrations of dimethyl sulfoxide between 1 and 20% v/v; propylene glycol, 1–50% v/v; glycerol, 1–20% v/v; dimethylformamide, 1–25% v/v; and Tween-30, 1–20% v/v. Inhibition by albumin was assayed at six concentrations between 0.5 and 5% w/v as final concentration in the assay solution and Luberol between 0.1 and 3% w/v as final concentration in the assay solution. From plots of inhibition vs. concentration for each solvent or emulsifier, the concentration required for 50% inhibition was determined. Each point on each curve was determined in duplicate, with a control from which UDPGA had been deleted.

Cation requirements for optimal conjugation of *p*-nitrophenol by the agarose-bound enzyme were studied using various concentrations of magnesium chloride and calcium chloride. These were added in the Tris buffer to the standard *p*-nitrophenol assay. The concentrations of *p*-nitrophenol, UDPGA, and agarose-bound enzyme were kept constant while each salt was studied at concentrations between 0.1 and 100 mM. Each point was determined in triplicate.

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References and Notes

(1) G. J. Dutton, Ed., "Glucuronic Acid, Free and Combined", Academic Press, New York, N.Y., 1966.
(2) S. Billets, P. S. Lietman, and C. Fenselau, *J. Med. Chem.*, **16**, 30–33 (1973).
(3) M. W. Anders and J. P. Latorre, *J. Chromatogr.*, **55**, 409–413 (1971).
(4) R. M. Thompson and D. M. Desiderio, *Biochem. Biophys. Res. Commun.*, **48**, 1303–1310 (1972).
(5) G. D. Paulson, R. G. Zaylskie, and M. M. Dockter, *Anal. Chem.*, **45**, 21–27 (1973).
(6) T. T. L. Chang, C. F. Kuhlman, R. T. Schillings, S. F. Sisenwine, C. O. Tio, and H. W. Ruelius, *Experientia*, **29**, 653–655 (1973).
(7) G. D. Paulson, A. M. Jacobsen, R. G. Zaylskie, and V. J. File, *Agric. Food Chem.*, **21**, 804–811 (1973).
(8) C. C. Porter, B. H. Arison, V. F. Gruber, D. C. Titus, and W. J. A. VandenHeuvel, *Drug Metab. Dispos.*, **3**, 189–197 (1975).
(9) I. Parikh, D. W. MacGlashan, and C. Fenselau, *J. Med. Chem.*, **19**, 296 (1976).
(10) L. Strebel and G. B. Odell, *Pediat. Res.*, **5**, 548–559 (1971).
(11) M. Salmon, C. Fenselau, J. O. Cukier, and G. B. Odell, *Life Sci.*, **15**, 2069–2073 (1974).
(12) C. Fenselau, S. Billets, and O. M. Colvin, Pharmacologic Studies Using Gas Chromatography–Mass Spectrometry, Tape Recording for Lab Professionals, American Chemical Society, 1973.
(13) D. A. Vessey, J. Goldenberg, and D. Zakim, *Biochim. Biophys. Acta*, **309**, 75–82 (1973).
(14) R. D. Howland, A. Burkhalter, A. J. Trevor, S. Hegeman, and D. Y. Shirachi, *Biochem. J.*, **125**, 991–997 (1971).
(15) S. C. March, I. Parikh, and P. Cuatrecases, *Anal. Biochem.*, **60**, 149–152 (1974).
(16) A. L. Mistra, R. B. Pontani, and S. J. Mule, *J. Chromatogr.*, **71**, 554–556 (1972).