maleic anhydride and 2.0 g. (10.7 mmoles) of ferrocene in 100 ml. of tetrahydrofuran was added 3 ml. of a 30% aqueous solution of hydrogen peroxide. The solution was then allowed to stand at room temperature for 60 hr. during which time it darkened and deposited a brown sludge. Next, 3 g. of anhydrous magnesium sulfate was added, and the resultant mixture was allowed to stand several hours. It was then filtered and evaporated to dryness on a rotary evaporator to yield 3.1 g. of a deep orange-blue solid. This material was transferred to a sublimator, unchanged ferrocene and maleic anhydride were sublimed, and a residue of 0.5 g. remained (27% yield based on maleic anhydride).

A benzene solution of the residue was chromatographed on deactivated silica gel. Ether–petroleum ether (b.p. 30–60°) (1:3) eluted a blue band which was evaporated to dryness to yield 0.2 g. (10%) of I as blue needles, m.p. 138–139.2° (recrystallized from hexane).

Anal. Calcd. for $C_{18}H_{16}FeO_4$: C, 61.4; H, 4.6; Fe, 15.8; mol. wt., 352. Found: C, 61.4; H, 4.6; Fe, 15.4; mol. wt., 360 ± 10 (ebullioscopic in benzene).

Methyl 6-*S*-Methyl-6-thiohexopyranosides. Effect of the Methylthio Group at C-6 on Rates of Enzymic and Nonenzymic Hydrolysis[1]

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We report the synthesis and certain properties of methyl 6-*S*-methyl-6-thio-β-D-glucopyranoside (I) and methyl 6-*S*-methyl-6-thio-α-D-galactopyranoside (II). These substances were prepared as glycosidase substrates and model compounds during an investigation of the mechanism of glycosidase–glycosyltransferase action.

Both syntheses involved conventional reactions. Compound I was prepared by the following scheme.

1,2-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-D-glucofuranose
↓ NaSMe
1,2-*O*-isopropylidene-6-*S*-methyl-6-thio-α-D-glucofuranose
MeOH ↓ HCl
I

Compound II was prepared by the sequence following.

1,2:3,4-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-galactose
↓ NaSMe
1,2:3,4-di-*O*-isopropylidene-6-*S*-methyl-6-thio-α-D-galactose
MeOH ↓ HCl
II

Configurations at the anomeric carbon of these glycosides were assigned on the basis of rotational data; the assignment of pyranoside structures was based on formic acid yields from periodate oxidation.

One objective in our general investigation of the mechanism of glycosidase–glycosyltransferase action has been to determine whether or not methylthio and other nucleophilic groups augment the rates of glycoside hydrolysis by neighboring-group assistance. Since the 1C and certain flexible conformations of methyl 6-*S*-methyl-6-thio-α-D-galactopyranoside allow proper orientation of the methylthio group for possible nucleophilic participation during hydrolysis,[3] the nonenzymic hydrolysis rate for this glycoside was determined. Hydrolysis rates for methyl 6-*O*-methyl-α-D-galactopyranoside and methyl α-D-galactopyranoside were determined under identical conditions for purposes of comparison. These rate determinations, although carried out by observing both the change in rotation of the reaction mixtures and the formation of reducing substances, leave something to be desired, since the vigorous conditions necessary to produce reasonable rates bring about some degradation of the reducing sugars. The hydrolysis data (shown in Table I)

TABLE I

RATES OF ACID-CATALYZED HYDROLYSIS OF GLYCOSIDES

Glycoside	Method	Temp., °C.	k_2, l. mole^{-1} sec.$^{-1}$
Methyl α-D-galactopyranoside	I[a]	60.0	1.0×10^{-5}
Methyl 6-*O*-methyl-α-D-galactopyranoside	I	60.0	5.4×10^{-6}
Methyl 6-*O*-methyl-α-D-galactopyranoside	II[b]	85.0	1.2×10^{-4}
Methyl 6-*S*-methyl-6-thio-α-D-galactopyranoside	I	60.0	6.7×10^{-6}
Methyl 6-*S*-methyl-6-thio-α-D-galactopyranoside	II	85.0	1.4×10^{-4}

[a] Polarimetric. [b] Colorimetric.

indicate no increase in rate on substituting a methylthio group for a hydroxyl group at C-6. Instead, the methyl 6-*S*-methyl-6-thio-α-D-galactopyranoside and methyl 6-*O*-methyl-α-D-galactopyranoside hydrolysis rates, which were nearly the same, appear to be significantly smaller than that for methyl α-D-galactopyranoside. Similar slowing of acetal and ketal hydrolysis on substituting methoxyl or methylthio at the γ-position (with respect to the potential carbonyl group) has been observed and attributed to a field effect.[4]

It is difficult to estimate—other than very roughly—the relative magnitudes of field effects by different C-6 substituents on these glycoside hydrolysis reactions. It seems reasonable to assume, however, that such an effect by a C-6 hydroxyl should be smaller than that by a methoxyl at this position; further, the effect by methoxyl should be somewhat smaller than that by methylthio—as has been observed in hydrolysis of 5-substituted 2-pentanone diethyl ketals.[4] The hydrolysis rate observed for compound II is slightly higher than that for the corresponding methoxy compound, but this may be a consequence of experimental error.

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(3) Although the C1 conformation is probably a lower energy conformation for α-D-galactopyranosides in solution, it is clear that the intermediacy of other conformations during reactions of these substances is by no means excluded. Pertinent to this matter is the alkaline degradation of phenyl β-D-galactopyranoside (presumably more stable in the C1 conformation than are the α-D-galactopyranosides), which proceeds by participation of oxygen at C-2 through either a half-chair or an approximately half-chair conformation to give phenoxide ion and 1,6-anhydro-β-D-galactopyranose as final products; pertinent also is the thermal degradation of D-lactose to 1,6-anhydro-β-D-galactopyranose.

(4) J. C. Speck, Jr., D. J. Rynbrandt, and I. H. Kochevar, *J. Am. Chem. Soc.*, **87**, 4979 (1965).

(5) *Cf.* H. Holtz and L. M. Stock, *ibid.*, **86**, 5188 (1964).

Compound I has been examined as a possible substrate for a β-D-glucosidase (sweet almond emulsin). The emulsin-catalyzed hydrolysis of this glycoside is at least 100 times as slow as that of methyl β-D-glucoside—in general agreement with earlier observations[6] on the effect of C-6 substituents on rates of emulsin-catalyzed hydrolysis of β-D-glucopyranosides. Compound I was tested also as a possible inhibitor of almond emulsin β-D-glucosidase activity and was found to be without inhibitory effect—even when the concentration was as high as that of the substrate, salicin, used in these experiments.

Experimental Section[7]

Materials.—1,2:3,4-Di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-galactose was prepared by a slight modification of the procedure given by Tipson.[8] 1,2-*O*-Isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose was prepared similarly except that the reaction with *p*-toluenesulfonyl chloride was carried out at 0°. 1,2:3,4-Di-*O*-isopropylidene-6-*O*-methyl-α-D-galactose was prepared according to the method of Goldstein, *et al.*[9] Methyl α-D-galactopyranoside was prepared by Dale and Hudson's procedure.[10] β-D-Glucosidase (sweet almond emulsin) was purchased as a partially purified preparation (minimum activity, 2.5 units/mg.) from Worthington Biochemical Corp. Salicin was obtained from Sigma Chemical Co. All other reagents either were reagent grade or were carefully purified before use.

1,2-*O*-Isopropylidene-6-S-methyl-6-thio-α-D-glucofuranose.—This substance was prepared by the same procedure as that employed for preparation of 1,2:3,4:di-*O*-isopropylidene-6-*S*-methyl-6-thio-α-D-galactose (see below) except that the reaction of the sodium salt of methanethiol with 1,2-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose was carried out in methanol solution. Purification of the product by short-path distillation at 110–120° (0.1 mm., bath temperature 150°) gave a nearly colorless syrup which usually crystallized within a short time if kept dry. Yields were 38–46%. Typical rotations of these preparations were $[\alpha]^{24}_{578}$ −7.6° (*c* 11.9, ethyl alcohol) and $[\alpha]^{26}_{578}$ −5.9° (*c* 8.09, ethyl alcohol), lit.[11] $[\alpha]^{23}_{D}$ −3.5°, −7.6° (*c* 2.3, ethyl alcohol).

1,2:3,4-Di-*O*-isopropylidene-6-*S*-methyl-6-thio-α-D-galactose.—A solution of methanethiol and its sodium salt in propyl alcohol was prepared by allowing 3.45 g. (0.15 g.-atom) of sodium to react with 200 ml. of dry propyl alcohol followed by saturating this solution with methanethiol (8.2 g. added). 1,2:3,4-Di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-galactose (37.9 g., 0.094 mole) was added to this solution, and the resulting mixture was boiled under reflux in an atmosphere of dry nitrogen for 3.5 hr. After cooling the mixture, the precipitate of sodium *p*-toluenesulfonate was filtered off, and excess sodium salt of methanethiol in the filtrate was decomposed by adding 2 ml. of water and saturating the solution with carbon dioxide. The precipitate formed during carbonation was removed by centrifuging, and propyl alcohol in the supernatant solution was evaporated at reduced pressure to give 20.6 g. (75%) of crude 1,2:3,4-di-*O*-isopropylidene-6-*S*-methyl-6-thio-α-D-galactose in the form of a light yellow syrup. Distillation of the crude product at 102–104° (0.3–0.5 mm., bath temperature 128°) gave a colorless syrup, $[\alpha]^{21}_{578}$ −72.4° (*c* 6.8, methanol).

Anal. Calcd. for $C_{13}H_{22}O_5S$: C, 53.77; H, 7.64; S, 11.04. Found: C, 53.87; H, 7.66; S, 11.11.

Methyl 6-*S*-Methyl-6-thio-β-D-glucopyranoside (I).—1,2-*O*-Isopropylidene-6-*S*-methyl-6-thio-α-D-glucofuranose was converted directly into I by dissolving 2.5 g. in 25 ml. of a 1% solution of hydrogen chloride in methanol and boiling the solution under reflux for 2 hr. On evaporating this solution to dryness at reduced pressure, the product crystallized from the concentrated solution. The crude glycoside was separated from some syrupy material in the residue by triturating with approximately 6 ml. of ethyl acetate and then with about 1.5 ml. of methanol: yield 0.45 g., $[\alpha]^{23}_{578}$ +13.0° (*c* 7.68, water). After one recrystallization from absolute ethyl alcohol, the rotation was $[\alpha]^{24}_{578}$ +5.85° (*c* 6.84, water), −5.4° (*c* 3.73, methanol). After three recrystallizations from absolute ethyl alcohol, the rotation was $[\alpha]^{21}_{578}$ −9.9° (*c* 2.52, methanol), m.p. 153.5–154.5°. Oxidation of approximately 0.05 g. of this glycoside in 20 ml. of 0.1 *M* neutral periodate for 9 hr. gave 90% of the theoretical amount of formic acid as measured by titration after reducing excess periodate with ethylene glycol.

Anal. Calcd. for $C_8H_{16}O_5S$: C, 42.84; H, 7.19; S, 14.30. Found: C, 43.18; H, 7.10; S, 14.30.

Methyl 6-*S*-Methyl-6-thio-α-D-galactopyranoside (II).—A solution of 11.6 g. (0.04 mole) of 1,2:3,4-di-*O*-isopropylidene-6-*S*-methyl-6-thio-α-D-galactose in 36 ml. of dry methanol containing 1.2% hydrogen chloride was heated in a sealed tube at 100° for 50 hr. Evaporation of this reaction mixture at reduced pressure gave a solid residue which was washed onto a filter with a little methanol and then washed with ethyl ether and dried: yield, approximately 5 g. This product was obtained as colorless platelets after two recrystallizations from absolute ethyl alcohol: m.p. 167–167.5°, $[\alpha]^{20}_{578}$ +156° (*c* 1.1, methanol). A third recrystallization from absolute ethyl alcohol did not change the rotation. The periodate oxidation of this glycoside was somewhat slow, producing 86% of the theoretical quantity of formic acid in 12 hr.

Anal. Calcd. for $C_8H_{16}O_5S$: C, 42.84; H, 7.19; S, 14.30. Found: C, 42.95; H, 7.07; S, 14.07.

Methyl 6-*O*-Methyl-α-D-galactopyranoside.—A solution of 7.0 g. (0.025 mole) of 1,2:3,4-di-*O*-isopropylidene-6-*O*-methyl-α-D-galactose in 30 ml. of methanolic hydrogen chloride (4% in hydrogen chloride) was heated in a sealed tube at 100° for 26.5 hr. The syrup obtained on evaporating this reaction mixture at reduced pressure crystallized when it was redissolved in methanol, and the resulting solution was treated with a small amount of Norit A, filtered, and then evaporated at reduced pressure. One recrystallization of this product from methanol followed by two recrystallizations from absolute ethyl alcohol gave 2 g. of methyl 6-*O*-methyl-α-D-galactopyranoside, $[\alpha]^{22}_{578}$ +173 (*c* 2.9, water), lit.[9] $[\alpha]^{20}_{D}$ +165 (*c* 1, water).

Rates of Nonenzymic Hydrolysis of Glycosides. A. Polarimetric Determinations.—These reactions were carried out at 60.0 ± 0.1° in 1.003 *M* hydrochloric acid. The initial glycoside concentration was approximately 0.05 *M*. For measurement of optical rotation the reaction mixture was transferred from the reaction vessel in the thermostat to a jacketed polarimeter tube maintained at 60.0°.

B. Determinations Based on Reducing Sugars Released.—The reaction conditions in these experiments were 85.0 ± 0.1°, 0.46 *M* perchloric acid, and an initial glycoside concentration of approximately 0.05 *M*. Aliquots (0.5 ml.) of the reaction mixture were removed at 0.5-hr. intervals and were neutralized immediately with 0.5 ml. of 0.5 *M* sodium hydroxide. Two milliliters of 3,5-dinitrosalicylate reagent (prepared by dissolving 10 g. of 3,5-dinitrosalicylic acid in 200 ml. of 2 *M* sodium hydroxide and diluting to 500 ml., dissolving 300 g. of potassium sodium tartrate in this solution, and diluting finally to 1 l.) was added to each neutralized aliquot. The mixture then was heated in a boiling-water bath for 5 min., cooled, and diluted to 25 ml., and the absorbance at 540 mμ was determined.

Determination of β-D-Glucosidase-Catalyzed Hydrolysis Rates.—For comparison of the relative rates of almond emulsin β-D-glucosidase-catalyzed hydrolysis of I and methyl β-D-glucopyranoside, reaction mixtures were made up in 0.08 *M*, pH 5.2 acetate buffer to a glycoside concentration of 0.10 *M* and an enzyme concentration of approximately 16.8 units of activity/ml. The reaction mixtures were incubated in a 25.0° thermostat, and 0.25-ml. aliquots were removed at 15-min. intervals over a period of 1 hr. for determination of released reducing sugar. Estimation of reducing sugars was carried out with 3,5-dinitrosalicylate reagent as described above.

For determining the extent of inhibition of almond emulsin β-D-glucosidase activity by I, the reaction mixtures were prepared as described in the preceding paragraph except that the highest concentration of I and of salicin, the substrate used in

(6) B. Helferich, S. Grünler, and A. Gnüchtel, *Z. Physiol. Chem.*, **248**, 85 (1937).

(7) Rotations were measured with a Zeiss photoelectric precision polarimeter. Melting points were determined in a Thomas–Hoover melting point apparatus. Melting points and boiling points are uncorrected. Microanalysis was carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(8) R. S. Tipson, *Methods Carbohydrate Chem.*, **2**, 248 (1963).

(9) I. J. Goldstein, J. K. Hamilton, and F. Smith, *J. Am. Chem. Soc.*, **79**, 1190 (1957).

(10) J. K. Dale and C. S. Hudson, *ibid.*, **52**, 2334 (1930).

(11) A. L. Raymond, *J. Biol. Chem.*, **107**, 85 (1934).

these experiments, was 0.035 *M*. Incubation of the reaction mixtures and determination of glucose released on hydrolysis were carried out as described above.

Acknowledgment.—The authors express their thanks to Mr. John N. LaRue for preparing methyl α-D-galactopyranoside and for one preparation of 1,2-*O*-isopropylidene-6-*S*-methyl-6-thio-α-D-glucofuranose, to Mrs. Irene H. Kochevar for checking certain rate determinations, and to Mr. George A. Stone for carrying out the β-D-glucosidase-catalyzed hydrolyses.

Proton Magnetic Resonance Spectra of Tetracyclines

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The investigational tool of n.m.r. spectroscopy has been applied rather infrequently to the solutions of problems posed by the chemistry of tetracyclines. In several instances, however, this method has been used successfully to clarify certain structural or stereochemical features of some tetracycline molecules.[1-7] Since the published data are few and dispersed widely, it is the intention of this paper to make available the information obtained in these laboratories by n.m.r. spectroscopy on a variety of tetracycline derivatives. The usefulness of these data has already been demonstrated in that they permitted structural, stereochemical, and conformational assignments hitherto not possible and it is anticipated that they will be of value in future investigations.

Generally, n.m.r. spectra of tetracyclines are complex and difficult to obtain for lack of suitable solvents. Several derivatives, however, are more amenable to this method and served as a starting point for our investigations.

Since the complete stereochemistry of oxytetracycline (**3**) has been elucidated recently,[5] it is now possible also to determine the conformations of several oxytetracycline derivatives in solution, a matter of interest particularly to those concerned with reaction mechanisms and drug–enzyme interactions. It now appears that the principal conformation for these derivatives is similar to that shown in Figure 1 for the parent compound. This is different from that derived tentatively from X-ray studies,[8] but it fulfills the following conditions: planarity of the two β-diketone systems C-11–C-12 and C-1–C-3, *trans*-diaxial relationship of the protons attached to C-4 and C-4a, and a pseudo-equatorial–pseudo-axial relationship of the two protons attached to C-5 and C-5a (*vide infra*). The conformation shown can roughly be described as holding the molecule in two plains, intersecting with each other at an angle of about 110° along a line connecting C-5 and C-12a. However, there is a certain amount of puckering of rings A and B.

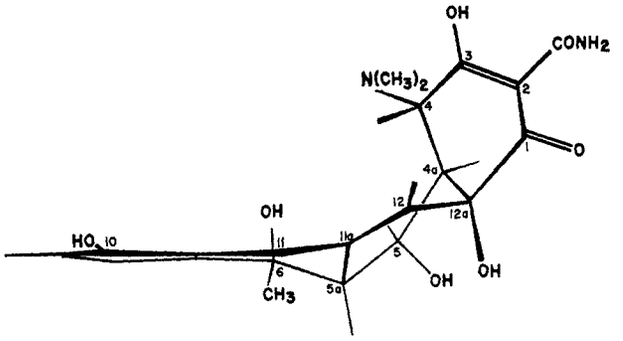
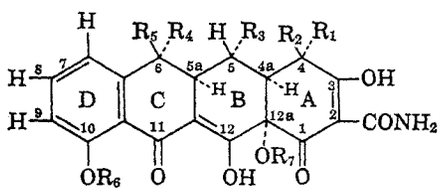
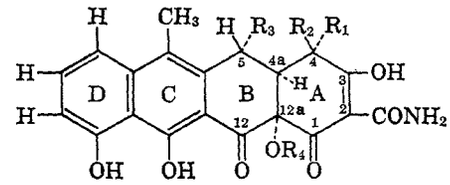


Figure 1.

The data shown in Table I were obtained in the solvents indicated, using tetramethylsilane as internal standard with a Varian A-60 instrument. When the chemical shift could not be determined unequivocally because of overlapping peaks, the range encompassing the signal under discussion is indicated. The coupling constants cited below were obtained directly from the spectra by simple first-order treatment.



	R1	R2	R3	R4	R5	R6	R7
1	$N(CH_3)_2$	H	H	CH_3	OH	H	H
2	H	$N(CH_3)_2$	H	CH_3	OH	H	H
3	$N(CH_3)_2$	H	OH	CH_3	OH	H	H
4	$N(CH_3)_2$	H	H	H	CH_3	H	H
5	$N(CH_3)_2$	H	OH	CH_3	H	H	H
6	$N(CH_3)_2$	H	OH	H	CH_3	H	H
7	$N(CH_3)_2$	H	H	CH_2		H	H
8	$N(CH_3)_2$	H	OH	CH_2		H	H
12	$N(CH_3)_2$	H	OH	CH_3	H	H	Ac
13	$N(CH_3)_2$	H	OAc	CH_3	OH	H	H
14	$N(CH_3)_2$	H	OAc	CH_3	OH	H	Ac
16	$N(CH_3)_2$	H	OH	CH_3	OH	Ac	Ac



	R1	R2	R3	R4
9	$N(CH_3)_2$	H	H	H
10	H	$N(CH_3)_2$	H	H
11	$N(CH_3)_2$	H	OH	H
15	$N(CH_3)_2$	H	OAc	Ac

The proton attached to C-4 gives a signal between 3.6 and 4 p.p.m. in pyridine solution, while in chloroform this peak shifts about 0.3 p.p.m. upfield. Protonation of the amino nitrogen causes this signal to shift downfield and in trifluoroacetic acid it generally falls

(1) M. Schach von Wittenau, F. A. Hochstein, and C. R. Stephens, *J. Org. Chem.*, **28**, 2454 (1963).

(2) C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. Schach von Wittenau, *J. Am. Chem. Soc.*, **85**, 2643 (1963).

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