enantiomorphs are listed the $2A$ and $2B$ values have been calculated. The $2A$ value represents the difference between, $2B$, the sum of, the molecular rotations of the α- and β-forms. The most striking fact brought out by Table I is that all of these glucoside tetrabenzoates are dextrorotatory, save that of cholesterol.

TABLE I

THE OPTICAL ROTATIONS OF SOME GLUCOSIDE 2,3,4,6-TETRABENZOATES

Aglucone	C_1 configuration	Optical rotation, in degrees $[\alpha]_D(CHCl_3)$	$2A$	$2B$
Methanol	α	$+84^a$	33,600	69,000
	β	$+29^a$		
n-Amyl alcohol	α	$+84^b$	44,000	67,000
	β	$+18^b$		
Cyclohexanol	α	$+84.5^b$	49,500	65,100
	β	$+11.5^b$		
Phenol	α	$+82^b$	36,500	73,500
	β	$+27^c$		
o-Nitrophenol	β	$+66^b$		
Cetyl alcohol	β	$+15.4^d$		
Cholesterol	β	-18.3^a		
β-Sitosterol	β	$+15.9^e$		
β-Sitosterol	β	$+18.3^d$		

[a] See footnote 6. [b] This work. [c] B. Helferich and F. Strauss, *J. prakt. Chem.*, **142**, 13 (1935). [d] A. H. Salway, *J. Chem. Soc.*, **103**, 1022 (1913). [e] L. J. Swift, THIS JOURNAL, **74**, 1099 (1952).

There are three glucoside pairs for which data are available for both anomers in the acetylated and benzoylated states. These are the methyl, cyclohexyl and phenyl glucosides. In each instance the $2A$ value is greater for the tetraacetate[13] than for the tetrabenzoate. This indicates that the aglucone has less influence upon the rotation of the benzoylated glucosides than upon that of the acetylated glucosides.

Pigman and Isbell found that the acetylated *aliphatic* glucosides had $2B$ values of approximately 40,000. For the acetylated *aromatic* glucosides the $2B$ values were higher, 62,000 in the case of the phenyl glucosides. As is shown by the table the $2B$ values for the benzoylated glucosides are higher ranging from 65,000 to 73,500, the highest value being that of the phenyl glucoside. The higher $2B$ values for the benzoylated glucosides indicate that the portion of the glucoside molecule composed of carbon atoms 2 to 6 is rendered more dextrorotatory by benzoylation than by acetylation.

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Structure of Galactosylglycerol from *Irideae laminarioides*

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The non-reducing galactoside is hydrolyzed with yeast α-galactosidase to yield D-galactose and glycerol. Complete methylation of the compound produces a hexamethylgalactoside, which on hydrolysis yields 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 1,3-di-*O*-methylglycerol. When the galactosylglycerol is oxidized with sodium periodate, two moles of periodate are consumed with the production of one mole of formic acid; no formaldehyde is produced. These results show that the structure of the compound is α-D-galactopyranosyl-2-glycerol.

A study of the carbohydrates that occur in the marine alga *Irideae laminarioides* revealed that this plant contains from 1 to 4% of an alcohol-soluble galactoside, consisting of galactose and glycerol. Colin and co-workers[1,2] previously showed that a galactosylglycerol (fluoridoside) occurs in many of the red algae. They isolated the compound and showed that it can be hydrolyzed with α-galactosidase, yielding equimolar quantities of D-galactose and glycerol. From this they concluded that the galactoside possessed an α-linkage. Colin[3] also showed that the galactoside was oxidized with difficulty by bromine, and that it was not attacked by *Acetobacter;* whereas the hydrolysis products were readily oxidized when treated with bromine or when inoculated with *Acetobacter*. On the basis of these results he concluded that the galactosidic linkage in the compound occurs through the secondary alcohol group of the glycerol.

Inasmuch as these data do not afford conclusive proof for the structure of this galactoside, we have undertaken a thorough investigation of its constitution, using the methylation and periodate oxidation procedures.

The galactoside was acetylated and the product methylated first with methyl sulfate and sodium hydroxide, and finally with methyl iodide and silver oxide. Methanolysis of the hexamethylgalactoside produced 2,3,4,6-tetra-*O*-methyl-D-galactose and 1,3-di-*O*-methylglycerol. These data, therefore, show that the galactose is linked through position 2 of glycerol.

Additional evidence for the support of the galactosylglycerol structure was obtained from periodate oxidation data on the original compound. On treatment of the galactosylglycerol with sodium periodate it consumed two moles of periodate and produced one mole of formic acid. No formaldehyde could be found in the reaction mixture. These data are consistent only with a structure in which the D-galactopyranosyl moiety is linked to the secondary alcohol of glycerol,

It can be concluded that the structure of the

(1) H. Colin and E. Guéguen, *Compt. rend.*, **191**, 163 (1930).
(2) H. Colin and J. Augier, *ibid.*, **195**, 1042 (1933).
(3) H. Colin, *Bull. soc. chim.*, [5] **4**, 277 (1937).

galactoside is α-D-galactopyranosyl-2-glycerol, as originally proposed by Colin,[3] and its formula can be written as shown in Fig. 1.

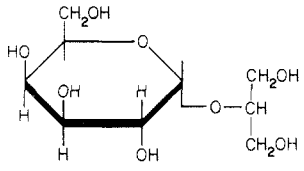

Fig. 1.

Experimental

Isolation of Crude Galactoside.—The *Irideae* plants were collected at Moss Beach, California. They were sun-dried and coarsely ground. A quantity of 1800 g. of the ground material was extracted three times with 3-liter portions of 80% alcohol on a steam-bath. The combined extracts were filtered and concentrated under reduced pressure. The salts, which were deposited from solution during concentration, were filtered off and the inorganic material was further eliminated by the use of ion-exchange columns (Duolite C-3 and A-3). Upon concentration of the neutral effluent 55 g. of an immobile sirup was obtained. On the basis of a specific rotation of +160° for the galactosylglycerol[3] the optical rotation of the sirup indicated that about 30 g. of the substance was present. The pure compound was isolated in crystalline form by first acetylating and then deacetylating the sirup, as suggested by Haas and Hill.[4]

Acetylation.—The sirup (55 g.) containing the galactoside was dissolved in 520 ml. of pyridine, the solution cooled to 2°, and 350 ml. of cold acetic anhydride was added slowly with stirring. The reaction mixture was kept at 2° for 24 hours, and then at room temperature for three days. The mixture was poured into approximately 3 liters of ice-water[5] with stirring. The acetylated product that crystallized after a short time was separated by filtration. After three recrystallizations of the crude product from 95% ethanol, a yield of 44.7 g. was obtained. The specific rotation of the acetylated galactoside (acetone, *c* 3) was $[\alpha]_D$ +114°; m.p. 101°. Its acetyl value[6] was 51.0%, which corresponds to a hexa-*O*-acetylgalactosylglycerol (calcd. CH_3CO for $C_5H_{12}O_5$-$(CH_3CO)_6$, 51.0%).

Deacetylation and Preparation of Crystalline D-Galactosylglycerol.—Twenty grams of the acetylated galactoside was deacetylated with barium methylate as described by Isbell.[7] The method was slightly modified by introducing the use of ion-exchange columns to eliminate residual inorganic impurities that remained after removal of the barium sulfate precipitate. The neutral effluent was concentrated to a thick sirup and the sirup was extracted with several portions of absolute alcohol. The combined alcoholic extract was concentrated under reduced pressure with occasional addition of benzene and absolute ethanol. In the course of concentration, crystalline material was deposited from solution. The crystals were collected on a büchner funnel and washed with cold absolute ethanol. The filtrate and washings were returned to the original flask and concentrated to yield a second crop of crystals. The crystalline material thus obtained was twice recrystallized from a minimum quantity of boiling absolute ethanol. A yield of 9.1 g. of anhydrous galactosylglycerol was obtained. The melting point of the compound was 128.5°. Its specific rotation in water (*c* 3.35) was $[\alpha]_D$ +165°.

Oxidation of the compound with nitric acid yielded mucic acid, showing the presence of galactose. On hydrolysis with acid the $[\alpha]_D$ of the material changed from +165° to +57°, the final value corresponding to the specific rotation of an equimolar mixture of D-galactose and glycerol. Paper chromatographic analysis products, using permanganate as a spray, indicated that the galactoside consists of galactose and glycerol. Hence the compound is a glycerol galactoside, When hydrolyzed with acid or with α-D-galactosidase, downward mutarotation was observed, indicating that the galactoside is of the α-type.

Methylation.—Fifteen grams of acetylated product was dissolved in 250 ml. of acetone in a 2-liter spherical flask. The flask was placed in a water-bath at 55° and the material was methylated with 120 ml. of methyl sulfate and 320 ml. of 30% sodium hydroxide. The reagents were added in 10 ml. equal portions at 10-minute intervals with vigorous mechanical stirring. After the reagents were added, stirring was continued for an hour. The flask with contents was then placed on a steam-bath, water was added to dissolve the solid sodium sulfate formed and the mixture maintained at 100° for about an hour. After cooling, the solution was extracted three times with chloroform and the combined chloroform extracts were concentrated to a thick sirup. This sirup was further subjected to three methylations with 120 ml. of methyl sulfate and 320 ml. of 30% sodium hydroxide by the above procedure. A yield of 9.4 g. of sirup was obtained. Attempts to determine its methoxyl content were not successful. Abnormally high methoxyl values were obtained, apparently due to the formation of volatile iodine compounds when the glycerol containing galactoside was treated with boiling hydriodic acid. This observation agrees with that of other investigators[8,9] that the presence of glycerol or glycerol-containing compounds causes high and erratic methoxyl values.

However, when a small sample of this sirup (50 mg.) was subjected to methanolysis followed by hydrolysis as later described, and the products examined by paper chromatography, using methyl ethyl ketone as a solvent[10] and *p*-anisidine hydrochloride as an indicator,[11] it was found that in addition to tetra-*O*-methyl-D-galactose, tri-*O*-methyl-D-galactose was present. This showed that the compound was not completely methylated.

A portion of the partially methylated product (3 g.) was further methylated as follows: The sirup was dissolved in 15 g. of methyl iodide in a three-necked flask equipped with a reflux condenser and a mercury seal stirrer. The methyl iodide was brought to a boil and 12 g. of silver oxide was added to the refluxing mixture in 1.2-g. portions every 30 minutes. After all of the silver oxide had been added, refluxing of the methyl iodide was continued for an hour. The contents of the flask were then extracted with ether, the extract filtered, the ether distilled off and the resulting sirup remethylated by the same procedure. A yield of 2.8 g. was obtained. The specific rotation of the sirup was $[\alpha]_D$ +156°.

Upon hydrolysis and chromatographic analysis of this sirup, tetra-*O*-methyl-D-galactose was obtained as the sole methylated galactose derivative. Furthermore, C and H analyses of the compound were consistent with the values calculated for tetra-*O*-methyl-galactosyl-di-*O*-methylglycerol. Thus, it was assumed that the galactoside was fully methylated.

Anal. Calcd. for $C_{15}H_{30}O_{14}$: C, 53.24; H, 8.94. Found: C, 53.04; H, 9.17; specific rotation, $[\alpha]_D$ +156° (in water, *c* 2.86).

Methanolysis and Hydrolysis of Tetra-*O*-methyl-galactosyl-di-*O*-methylglycerol.—Three tubes, each containing 0.6 g. of the methylated product and 10 ml. of 1 *N* methanolic hydrochloric acid, were sealed and kept at 100° for six hours. The tubes were opened, the methanol boiled off and a similar amount of 1 *N* hydrochloric acid was added to each. The tubes were resealed and heated again for 3 hours at 100°. They were then opened and neutralized with solid silver carbonate. The silver chloride was filtered off, and the filtrate transferred to a still equipped with a Vigreux column. After distilling off the water at atmospheric pressure, the residue was dissolved in ethyl ether, the solution transferred to a small vacuum still and the ether distilled off at room temperature, using a water aspirator. When the bath temperature was raised to 75°, 0.6 g. of a colorless, mobile liquid (fraction a) distilled over, leaving a residue (fraction b) in the vacuum still. Subsequent analysis proved fraction a to be 1,3-di-*O*-methylglycerol, and fraction b, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose.

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Identification of 1,3-Di-*O*-methylglycerol.—Utilizing the procedure described by Fairbourne[12] for the preparation of crystalline derivatives of methyl-*O*-glycerols, 0.36 g. of fraction a was warmed with 0.5 ml. of pyridine and 0.60 g. of *p*-nitrobenzoyl chloride dissolved in 3 ml. of chloroform was added. After allowing the reaction mixture to stand for 48 hours, 10 ml. of water was added and the mixture extracted with 10 ml. of ethyl ether. The ether extract was washed with water, dilute sulfuric acid, dilute sodium bicarbonate and finally with water again. The extract was dried over calcium chloride and concentrated under reduced pressure to yield a sirup which readily crystallized when triturated. When this product was twice recrystallized from petroleum ether, crystalline flat plates were obtained which melted at 40°, a value in agreement with that of the *p*-nitrobenzoic acid ester of dimethyl-*O*-glycerol.

Anal. Calcd. for $C_{12}H_{15}O_6N$: C, 53.53; H, 5.58; N, 5.20. Found: C, 53.66; H, 5.63; N, 5.15.

Measurement of the specific rotation of fraction a, both as pure substance and in aqueous solution gave a value of $[\alpha]_D$ 0, indicating that the compound is symmetrical and therefore must be the 1,3-di-*O*-methyl derivative of glycerol. The asymmetric D- and L-isomers of 1,2-di-*O*-methylglycerol prepared by Baer and Fischer[13] possess a measurable optical activity.

As confirmation, authentic 1,3-di-*O*-methylglycerol was prepared by the action of sodium methoxide on dibromohydrin.[14] The product obtained distilled at the same temperature (75°) as the dimethylglycerol derived from the methylated galactoside and when treated with *p*-nitrobenzoyl chloride, as previously described, produced a crystalline derivative with a melting point of 40°. When these crystals were mixed with those of the *p*-nitrobenzoyl acid ester of the dimethylglycerol derived from the galactosylglycerol, no depression of melting point could be observed. The dimethylglycerol derived from the galactoside is therefore the 1,3-di-*O*-methylglycerol.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-galactose.—The residue in the vacuum still (fraction b) was extracted with ethyl ether, the extract treated with decolorizing carbon, and concentrated to a small volume. Upon the addition of an equal volume of petroleum ether to the extract, a turbidity appeared in the solution. The tube containing the mixture was placed in a bath at 35° until the solution had cleared, and was then placed in a −20° chamber. After 24 hours crystals began to deposit, yielding about 0.6 g. of colorless fine needles. The product cochromatographed with authentic 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, using methyl ethyl ketone as solvent, and gave the theoretical methoxyl value of 52.5%. The initial specific rotation of the tetra-*O*-methylgalactose (in water, *c* 3) was $[\alpha]_D$ +150°, mutarotating downward to +114°, which indicated that it crystallized in the α-form.[15,16] Its melting point was 69°.

The anilide of the 2,3,4,6-tetra-*O*-methylgalactose was prepared by dissolving a 0.3-g. sample in 10 ml. of ethanol, containing 0.12 g. of aniline and a trace of hydrochloric acid. The mixture was refluxed for 3 hours and then cooled to −20°. Crystallization was instantaneous, yielding needles which after recrystallization from alcohol had a specific rotation in pyridine (*c* 0.5) of $[\alpha]_D$ −140°, and in acetone (*c* 1), $[\alpha]_D$ −84°.[15]

Its melting point was 197°, and was not changed when mixed with an authentic sample of 2,3,4,6-tetra-*O*-methyl-D-galactose anilide.

Anal. Calcd. for $C_{16}O_5H_{25}N$: C, 61.74; H, 8.05; N, 4.50; OCH_3, 39.87. Found: C, 62.08; H, 8.16; N, 4.49; OCH_3, 39.9.

Periodate Oxidation.—A 0.5 m*M* (127 mg.) sample of the galactosylglycerol was dissolved in 25 ml. of water, 5 ml. of 0.4 *M* sodium periodate was added and the mixture was diluted to 50 ml. A blank containing the reagents, but no galactoside was used in the determination of the reduced periodate. Another blank, to which 2.5 ml. of ethylene glycol had been added prior to the addition of the sodium periodate, served in the determination of formic acid. After allowing the solutions to remain at room temperature in the dark for 24 hours, the samples were analyzed. For the determination of formic acid, 5-ml. aliquots of the oxidized mixture were taken, treated with 0.25 ml. of ethylene glycol and after 5 minutes were titrated with 0.01 *N* sodium hydroxide, using phenol red as an indicator. The amount of periodate consumed in the reaction was estimated by treating 5-ml. samples with an excess of 0.1 *N* sodium arsenite in the presence of potassium iodide and sodium bicarbonate buffer and back-titrating with 0.1 *N* iodine.

The results showed that two moles of periodate were consumed, giving rise to one mole of formic acid in the oxidation of one mole of galactosylglycerol. No formaldehyde could be detected in the oxidation mixture.[17]

These data confirm the structure of the galactoside as α-D-galactopyranosyl-2-glycerol.

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