a colorless viscous oil, whose infrared spectrum (OH absorption at 3350 cm.$^{-1}$) was identical with authentic material.[16]

(e) Benzoic Acid.—The radioactive 1-phenylcycloheptanol (56 mg.) was refluxed with potassium permanganate (210 mg.) in water (20 ml.) for 16 hr. The solution was filtered and the acidified filtrate extracted with ether. The dried ether extract was evaporated and the residue sublimed *in vacuo*. The white sublimate was crystallized from hot water yielding colorless plates of benzoic acid (6.3 mg.), m.p. 121–122°, not depressed on admixture with an authentic specimen.

The specific activities of the degradation products of the hyoscyamine are recorded in Table I, corrected for the various dilutions which were made in the course of the degradation.

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The Plant Sulfolipid. VI. Configuration of the Glycerol Moiety[1]

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The D-configuration of the glycerol in 6-sulfo-α-D-quinovopyranosyl-(1→1)-glycerol (I) derived from the plant sulfolipid has been demonstrated by radiochemical techniques. The same configuration was observed in the glycerol moieties of β-D-galactopyranosyl-(1→1)-glycerol (IIa) and α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→1)-glycerol (IIb) obtained from the galactosyl diglycerides. C^{14}-Labeled glycosyl-(1→1)-glycerols were oxidized with nitrogen dioxide. Glyceric acid-C^{14} was isolated by two-dimensional paper chromatography of acid hydrolysates of the oxidation products. It was co-crystallized with salts of D-, L- and DL-glyceric acids. In each case the specific activity of the L-glyceric acid salt was undiminished by recrystallization. The major glycolipids in plants, therefore, are glycosyl-(1→1′)-2′,3′-diacyl-D-glycerols.

The major lipids of chloroplasts are the galactosyl diglycerides.[4,5] The configuration of the glycerol moiety in these compounds is indicative of specificities involved in phytosynthesis of triglycerides. The most abundant, O-β-D-galactopyranosyl-(1 → 1)-glycerol and O-α-D-galactopyranosyl-(1 → 6)-O-β-D-galactopyranosyl-(1 → 1)-glycerol were first obtained by Carter, McCluer and Slifer,[4] by deacylation of wheat flour lipids. The third most abundant glycolipid in photosynthetic tissues is a sulfolipid with a structure similar to those of the galactolipids. Its deacylation product has been characterized and shown to be 6-sulfo-O-α-D-quinovopyranosyl-(1 → 1)-glycerol (I).[6] These glycolipids are formed concurrently[7] and it is of interest to consider the nature of the diglyceride pools involved in their biosynthesis. The small rotatory contribution of an asymmetric glycerol in these compounds precludes assignment of its configuration based upon rotations of the glycosides.

The availability of C^{14}-labeled glycosylglycerols by deacylation of the lipid products of photosynthesis[7] in $C^{14}O_2$ has made possible a re-evaluation of these structural relationships. This paper reports results of comparison of the configuration of the glycerol moieties in these compounds with those of the authentic asymmetric glyceric acids.

Discussion

O-β-Galactopyranosyl-(1 → 1)-glycerol (G–Gal) (IIa) and O-α-D-galactopyranosyl-(1 → 6)-O-β-D-galactopyranosyl-(1 → 1)-glycerol (G-Gal-Gal) (IIb) were used for model experiments to establish the method. Carter, *et al.*,[4] determined the structure of the G-Gal (m.p. 139–140°, $[\alpha]_D$ +3.77°) and G-Gal-Gal (m.p. 182–184°, $[\alpha]_D$ +86.4°) obtained from wheat flour lipids, except for the configuration of glycerol moiety. G-Gal-Gal (m.p. 196–198°, $[\alpha]^{23}_D$ +88°) was isolated later by Wickberg[8] from the red algae *Polysiphonia fastigiata* and *Corallina officinalis*. It was hydrolyzed to G-Gal with α-galactosidase from a yeast hexokinase preparation. Wickberg synthesized β-D-galactopyranosyl-(1 → 1)-D- (m.p. 140.5–141.5°, $[\alpha]^{20}_D$ −7°) (IIa) and-L-glycerol (m.p. 97–100°, $[\alpha]^{18}_D$ +1°) and concluded from mixed melting point measurements[9] that the naturally occurring G-Gal and G-Gal-Gal possess the D-glycerol structure, although the optical rotation of the natural G-Gal suggested, with less certainty, an L-glycerol structure.

Radiochemical analysis of C^{14}-labeled G-Gal and G-Gal-Gal carried out in this Laboratory has confirmed Wickberg's conclusions. G-Gal obtained by two-dimensional paper chromatography of C^{14}-labeled *Chlorella pyrenoidosa* lipid hydrolysates was oxidized with nitrogen dioxide in dry carbon tetrachloride to a mixture of nitrites of III which was then hydrolyzed with hydrochloric acid and chromatographed to obtain C^{14}-labeled glyceric acid in 36% yield. The small yield (7.3% of the calculated value) of glycolic acid probably was formed by decarboxylation of hydroxymalonic acid, an oxidation product of glycerol. Repeated recrystallizations of authentic asymmetric glyceric acid salts were carried out in the presence of the C^{14}-

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TABLE I

DETERMINATION OF GLYCEROL CONFIGURATION IN DEACYLATION PRODUCTS OF THE PLANT SULFOLIPID AND GALACTOLIPIDS

Source of glyceric acid-C^{14}	Authentic crystalline glyceric acid salt, mg.			Total C^{14}-activity used, c.p.m.	Times recrystallized	Measured specific activity, c.p.m./mg.	% C^{14} retention observed[g]
G-Gal[a]	Ca	DL(±)	33.283	32,400	2	957	98.1
G-Gal	Ca	L(+)	23.995	32,400	2	1360	100
G-Gal	Ca	L(+)	39.037	32,400	2	839	102
G-Gal	Ca	D(−)	19.246	32,400	2	213	12.6
G-Gal	Quinine	D(−)	26.443	32,400	2	100	8.2
G-Gal[b]	Quinine	D(−)	9.390	50,000	2	1430	26.8
G-Gal	Ca	L(+)	18.607	50,000	2	1770	65.8
G-Gal	Quinine	D(−)	21.589	66,700	1	1090	35.1
G-Gal	Ca	L(+)	34.031	133,000	1	3070	78.1
G-Gal-Gal	Ca	L(+)	24.639	32,500	2	1250	94.1
G-Gal-Gal	Ca	L(+)	24.639[c]	32,500	3	1180	88.3
G-Gal-Gal	Quinine	D(−)	24.924	32,500	2	523	39.9
G-Gal-Gal	Quinine	D(−)	24.924	32,500	3	237	18.2
G-Gal-Gal	Ca	DL(±)	25.896	19,050	2	750	101
G-Gal-Gal	Ca	D(−)	14.268	19,050	2	276	20.7
G-Gal-Gal	Ca	L(+)	25.508	19,050	1	865	115
G-Gal-Gal	Quinine	D(−)	19.127	19,050	2	179	17.9
G-Quin-SO_3H	Ca	L(+)	26.8	3,315	2	161	130
G-Quin-SO_3H	Ca	L(+)	26.8	3,315	3	141	110
G-Quin-SO_3H	Quinine	D(−)	33.4	3,315	2	26	25
G-Quin-SO_3H	Quinine	D(−)	33.4	3,315	4	7.5	7.6
G-Quin-SO_3H	Quinine	D(−)	33.4	3,315	5	0	2.5
Wheat-C^{14}[d]	Ca	L(+)	42.2	4,500	1	16	15
Wheat-C^{14}	Quinine	D(−)	35.2	(11,000)[e]	2	326	...
Wheat-C^{14}	Quinine	D(−)	35.2	(11,000)[e]	4	346	...
Wheat-C^{14}	Quinine	D(−)	35.2	(11,000)[e]	5	345	...
Glycolic acid-C^{14}[f]	Quinine	D(−)	27.593	32,800	2	139	11.7
Glycolic acid-C^{14}	Ca	L(+)	26.837	43,700	2	148	9.0

[a] One-hour acid hydrolysis of nitrogen dioxide oxidation products. [b] Two-hours acid hydrolysis of oxidation products. [c] Recrystallization of same sample. [d] Free glyceric acid-C^{14} isolated by paper chromatography of $C^{14}O_2$ photosynthesis products from wheat leaves. [e] Approximate figure. [f] Glycolic acid-C^{14} eluted from chromatograms of hydrolysates of oxidation products of G-Gal-C^{14}. [g] % Retention observed = 100 × (specific C^{14} activity) (wt. of authentic crystalline glyceric acid salt)/(total C^{14} activity used).

labeled glyceric acids derived from each of the glycolipids. The fact that radioactivity was retained quantitatively in calcium DL-glycerate and in calcium L(+)-glycerate and not in the quinine salt of D(−)-glyceric acid clearly indicated that the glyceric acid-C^{14} possessed the L-configuration (IV) (Table I). Therefore, the assignment of the D-glycerol configuration in G-Gal was shown to be correct.

Configuration of Sulfoquinovosylglycerol.—The method described above was successfully applied for elucidation of the glycerol configuration in sulfoquinovosylglycerol, G-Quin-SO_3H. As shown in Table I, G-Quin-SO_3H-C^{14} obtained by hydrolysis of the sulfolipid-C^{14} yielded pure L(+)-glyceric acid; therefore G-Quin-SO_3H has the D-glyceryl structure, I. This result provides evidence that the radiochemical analysis is generally applicable for glyceryl glycosides regardless of the anomeric structure of the glycoside component. It was envisioned also that the phytosyntheses of G-Gal, G-Gal-Gal and G-Quin-SO_3H lipids have some features in common, and that naturally occurring diglycerides probably are asymmetric.

It is apparent that phytosynthesis of the glycolipids utilizes a common diglyceride pool. The asymmetry of the diglyceride moiety of the glycolipids may arise in either of two ways. The diglyceride pool may be asymmetric, consisting of 2,3-diacyl-D-glycerol, or the glycosylation of diglycerides may be stereospecific for these diglycerides. The rapid turnover of galactolipids during photosynthesis within the chloroplast[7] suggests that the asymmetry in the products of this active metabolism may lead to asymmetry in triglycerides in Nature.

Experimental Part

C^{14}-Labeled Glyceric Acid from G-Gal-C^{14}.—G-Gal-C^{14} was isolated by two-dimensional paper chromatography of the deacylation products of *Chlorella* lipids labeled during 5 days photosynthesis in $C^{14}O_2$. The G-Gal-C^{14} (97,000 c.p.m.) was eluted from the paper with water, evaporated to dryness with an air stream and treated for 5 days at room temperature with 0.5 ml. dry carbon tetrachloride saturated with nitrogen dioxide in a Teflon-capped vial. The reagent and solvent were evaporated and the residue heated with 0.1 ml. of 2 *N* hydrochloric acid at 100° for 1 hour. The acid was evaporated with air and the residue taken up in a minimum volume of water and chromatographed[10] in phenol–water (PW) (100:39 w./w.) and in 1-butanol–propionic acid–water (142:71:100 v./v.) (BPAW) solvents. The labeled product (11,600 c.p.m.) having chromatographic coördinates of glyceric acid[10] represented a 36% yield. Glycolic acid was also isolated (7.3% yield).

Another sample of G-Gal was oxidized similarly and the products hydrolyzed in acid for 2 hours at 100°. The yield of glyceric acid was 22%. It was found to be partially racemized, presumably because of the increased time of acid hydrolysis.

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C^{14}-Labeled Glyceric Acid from G-Gal-Gal-C^{14}.—From 340,000 c.p.m. of G-Gal-Gal isolated in the same chromatograms as the G-Gal above, was obtained 11,000 c.p.m. (9.6% yield) of glyceric acid C^{14}. The time of hydrolysis in hydrochloric acid was 2 hours.

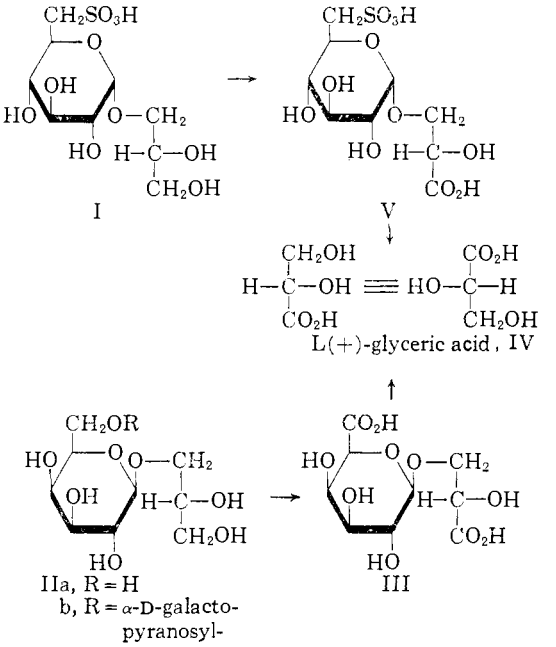
C^{14}-Labeled Glyceric Acid from G-Quin-SO_3H-C^{14}.—G-Quin-SO_3H eluted from the same chromatograms was oxidized as above with nitrogen dioxide, hydrolyzed for 2 hours at 100° with *N* hydrochloric acid. The chromatographically purified glyceric acid was co-crystallized with authentic enantiomorphic salts.

Authentic Crystalline Glyceric Acid Salts.—DL-Glyceric acid was prepared by nitric acid oxidation of glycerol,[11,12] and converted to the quinine salt for resolution.[13] Direct preparation of optically active glyceric acids from the serines[14] was found to be less effective.

Co-crystallization of Glyceric Acids-C^{14} with Authentic Enantiomorphs.—Radioactive glyceric acids were eluted with water from the paper chromatograms to provide standard solutions. Portions (25 μl.) of these were transferred to 25-ml. glass vials in which they were dried and taken up in 75 μl. of 50% ethanol. Dowex-50 (*ca.* 25 mg.) was added to each; the solutions were dried and taken up in 2 ml. of 95% ethanol. Scintillation liquid, 18 ml. (5 g. of PPO and 100 mg. of POPOP per liter of toluene[15]), was added and the samples counted in the Packard Tri-carb[15] liquid scintillation counter. Background counts were made using identical preparations.

Samples of the glyceric acids-C^{14} (*ca.* 100 μl.) containing known amounts of C^{14} were added to *ca.* 20-mg. samples of the authentic crystalline salts in 3-ml. conical centrifuge tubes. The mixture was recrystallized two to five times from hot water with addition of some ethanol when necessary. After removal of mother liquors with a capillary dropper, the salt was dried *in vacuo* and small samples weighed in the counting vials for determination of specific activities. The samples were treated with Dowex-50 in 75 μl. of 50% ethanol and dried. Radioactivity was measured after the addition of 2 ml. of 95% ethanol and 18 ml. of scintillation solution. Results are given in Table I.

To ensure reliability of the method, glycolic acid-C^{14} activity was co-crystallized with D- and L-glyceric acid salts. No radioactivity was retained. Glyceric acid-C^{14}, obtained from chromatograms of photosynthetic products of wheat leaves, co-crystallized quantitatively with D(−)-glyceric quinine salt. The radioactivity was not retained in calcium L(+)-glycerate.



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The Plant Sulfolipid. VII. Synthesis of 6-Sulfo-α-D-quinovopyranosyl-(1→1′)-glycerol and Radiochemical Syntheses of Sulfolipids[1]

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The synthesis of 6-sulfo-O-α-D-quinovopyranosyl-(1→1′)-glycerol and its identity with the sulfoglycosyl glycerol obtained by deacylation of the plant sulfolipid are reported. 6-Sulfo-quinovose was prepared from 1,2-isopropylidene-6-O-tosyl-D-glucofuranose by sulfite replacement. It was converted to the allyl α-glycoside which yielded the desired sulfoquinovosyl glycerol upon oxidation by permanganate. Similarly, S^{35}-labeled sulfoquinovose was converted to the allyl glycoside and to the corresponding dibromide. Radiochemical syntheses of S^{35}-labeled sulfolipids and transglycosidation reactions of 6-sulfo-D-quinovosides-S^{35} are described.

Introduction

The sulfolipid occurring in photosynthetic tissues was presumed to be a sulfoglycosyl glyceride[4] on the basis of radiochromatographic evidence. Mild saponification of the sulfolipid yielded a sulfoglycosylglycerol which was isolated as a crystalline salt after purification by anion exchange resin chromatography.[5] The sulfosugar liberated by acid hydrolysis of the sulfoglycosylglycerol was recently identified[6] as 6-sulfo-D-quinovose (6-deoxy-D-glucose-6-sulfonic acid) (I). The α-glucosidic linkage in the natural glycoside was also demonstrated.

In this paper are described syntheses of 6-sulfo-D-quinovose,[6a] 6-sulfo-O-α-D-quinovopyranosyl-(1 → 1)-glycerol and S^{35}-labeled sulfolipid which are the first carbohydrates containing the sulfonic acid group to be recognized in Nature.

(1) This work was supported by Grants A-2567 from the National Institute for Arthritis and Metabolic Diseases of the Public Health Service, the National Science Foundation, the Atomic Energy Commission and the Pennsylvania Agricultural Experiment Station.

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