Long-chain cyclic acetals of glycerol: metabolism of the stereomeric 1,3-dioxanes and 1,3-dioxolanes in myelinating rat brain

K. L. Su, W. J. Baumann, T. H. Madson, and H. H. O. Schmid

University of Minnesota, The Hormel Institute, Austin, Minnesota 55912

Abstract The metabolism of the stereomeric cyclic glycerol acetals of [1-^{14}C]hexadecanal was studied in myelinating rat brain. It was found that the four isomers, *cis*- and *trans*-2-pentadecyl-5-hydroxy-1,3-dioxanes and *cis*- and *trans*-2-pentadecyl-4-hydroxymethyl-1,3-dioxolanes, were utilized by the tissue at different rates. The acetals were primarily metabolized via a ring-opening mechanism leading to palmitic acid, some of which was subsequently elongated–desaturated. Only the five-membered ring isomers were incorporated as intact acetals into both neutral and polar brain lipids.

Supplementary key words 2-pentadecyl-5-hydroxy-1,3-dioxane · 2-pentadecyl-4-hydroxymethyl-1,3-dioxolane · enzymic acetal cleavage

The long-chain cyclic acetals of glycerol found in lipid extracts are usually considered artifacts that are produced during hydrolysis of lipid constituents containing the alk-1-enyl glycerol backbone (1–6). There exists evidence also that cyclic glycerol acetals occur as genuine lipid constituents, at least in some marine species (7–9). More recently, smooth muscle-stimulating properties were ascribed to a long-chain cyclic acetal of glycerophosphate (10). Metabolic studies with long-chain cyclic acetals of glycerol as substrates have not been reported, although preliminary evidence has been presented that in the digestive gland of starfish, glycerol acetals may be intermediates in plasmalogen biosynthesis (9).

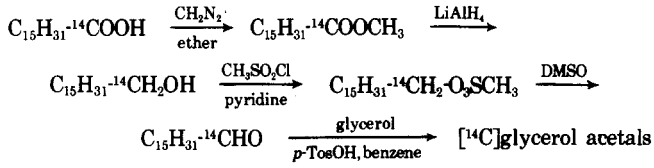
Our recent work (11, 12) on the formation and conformation of the stereomeric cyclic acetals of glycerol (Fig. 1) enabled us to prepare individual glycerol acetals in labeled form and to use them in metabolic studies.

In the present communication we report the metabolic behavior of the four isomeric cyclic glycerol acetals of [1-^{14}C]hexadecanal in mammalian brain. Myelinating rat brain was chosen because of its high rate of phospholipid biosynthesis (13) and because of the extensive information available on the metabolism of other intracerebrally administered long-chain precursors (14–22). The experiments were designed to determine (*a*) whether intact long-chain acetals of glycerol are incorporated into neutral and polar lipids, (*b*) to what degree the isomers are catabolized, and (*c*) whether glycerol acetals can be converted to alk-1-enyl ethers of glycerol in myelinating rat brain.

MATERIALS AND METHODS

Materials

[1-^{14}C]Palmitic acid (58 mCi/mmole, Dhom Products, Ltd., North Hollywood, Calif.) was converted to [1-^{14}C]hexadecanal via the methyl ester (23), hexadecanol (24), and hexadecyl methanesulfonate (25), followed by dimethyl sulfoxide oxidation (26), in an overall yield of 24%.



The intermediate methanesulfonate and [1-^{14}C]hexadecanal were purified by TLC to a radiopurity of >99%; the developing solvents were benzene and hexane–diethyl ether 90:10 (v/v), respectively. *p*-Toluenesulfonic acid-catalyzed condensation of [1-^{14}C]hexadecanal with glycerol led quantitatively to a mixture of the four isomeric C_{16} glycerol acetals (11). The acetal mixture was acetylated and then fractionated by TLC to yield the acetates of the individual *cis*- and *trans*-1,3-dioxanes and of a mixture of *cis*- and *trans*-1,3-dioxolanes; the developing sol-

Abbreviations: the four isomeric cyclic glycerol acetals are designated as follows: *cis*-6 stands for *cis*-2-pentadecyl-5-hydroxy-1,3-dioxane, *trans*-6 for *trans*-2-pentadecyl-5-hydroxy-1,3-dioxane, *cis*-5 for *cis*-2-pentadecyl-4-hydroxymethyl-1,3-dioxolane, and *trans*-5 for *trans*-2-pentadecyl-4-hydroxymethyl-1,3-dioxolane; structures of the isomers are given in Fig. 1. TLC, thin-layer chromatography; GLC, gas–liquid chromatography; PL, polar lipids; NL, neutral lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; *p*-TosOH, *p*-toluenesulfonic acid; DMSO, dimethyl sulfoxide.

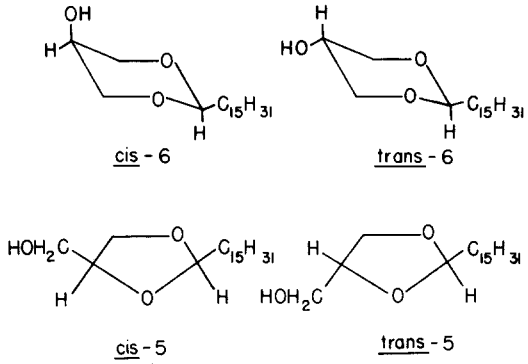


Fig. 1. The stereomeric glycerol acetals.

vent was hexane–diethyl ether 70:30 (v/v). The purity of each fraction was established by TLC and by GLC (11). The fractions were deacetylated by $LiAlH_4$ reduction (11) and finally purified by TLC to a radiopurity of >99%; the developing solvent was hexane–diethyl ether 40:60 (v/v).

Methods

Emulsions of cyclic glycerol acetals in sodium choleate were administered intracerebrally (14) to 18-day-old male albino rats (Sprague-Dawley strain, Dan Rolfsmeyer Co., Madison, Wis.). The rats, in groups of six, were killed by decapitation after 6 or 24 hr; the brains within each group were pooled, and the lipids were extracted as described previously (14).

Small aliquots of the total lipids were used to determine the recovery of radioactivity and the incorporation of radioactivity into individual lipid fractions. The lipids were resolved by TLC on layers, 0.5 mm thick, of Silica Gel H (Merck), using hexane–diethyl ether 50:50 (v/v) and 90:10 (v/v) and chloroform–methanol–water 65:25:4 (v/v/v) as developing solvents.

Neutral lipids were isolated from unused labeled substrate and polar lipids by preparative TLC in hexane–diethyl ether 90:10 (v/v), and the unused substrate was removed from the polar lipids by TLC in hexane–diethyl ether 50:50 (v/v). Polar and neutral lipids were then subjected to hydrogenolysis with $LiAlH_4$ in anhydrous diethyl ether (27) followed by hydrolysis with water. The distribution of radioactivity in the reduced products was examined after TLC in hexane–diethyl ether 40:60 (v/v), developed twice.

GLC analyses and preparative GLC were done on a Victoreen 4000 instrument equipped with flame ionization and thermal conductivity detectors. The aluminum column, 180 cm × 0.4 cm ID, was packed with 18% ethylene glycol succinate (Hi-Eff-2BP) on Gas-Chrom P, 80–100 mesh (Applied Science Laboratories, Inc., State College, Penn.). The column was operated at 190°C for the fractionation of alkyl acetates (14) and at 215°C for the fractionation of the acetates of glycerol acetals (11, 12).

Radioactivities were measured with a Packard Tri-Carb scintillation spectrometer (counting efficiency 80–81%) in a 0.55% (w/v) solution of Permablend I (Packard) in toluene.

TABLE 1. Incorporation of a mixture of isomeric C_{16} cyclic acetals of glycerol into rat brain lipids

	Time	
	6 hr	24 hr
Total radioactivity injected (cpm × 10^{-5} per brain)	18.2	18.2
Total radioactivity recovered (cpm × 10^{-5} per brain)	7.47	4.20
Percent recovered	41.1	23.1
Distribution of radioactivity (%)		
Substrate recovered	79.4	36.7
Polar lipids	18.4	58.7
Neutral lipids	2.2	4.6

TABLE 2. Distribution of radioactivity among the isomeric C_{16} cyclic acetals of glycerol administered and recovered

Acetal Isomer	Administered	Recovered after	
		6 hr	24 hr
	%	%	%
cis-6	36.1	41.3	45.7
trans-6	16.6	20.9	17.8
cis-5	28.7	24.0	19.2
trans-5	18.6	13.8	17.3

RESULTS AND DISCUSSION

The purpose of a preliminary experiment was to determine whether, in myelinating rat brain, long-chain cyclic acetals of glycerol are catabolized and whether they are

TABLE 3. Incorporation of the isomeric C_{16} cyclic acetals of glycerol into rat brain lipids (12 hr)

	Glycerol Acetal Administered		
	cis-6	*trans*-6	*cis*/*trans*-5[a]
Total radioactivity injected (cpm × 10^{-5} per brain)	10.0	10.7	16.7
Total radioactivity recovered (cpm × 10^{-5} per brain)	2.58	2.87	3.95
Percent recovered	25.8	26.8	23.6
Distribution of radioactivity (%)			
Substrate recovered	68.9[b]	44.3[b]	47.2[c]
Polar lipids	28.4	53.4	49.0
PE fraction	28.2	31.5	27.1
PC fraction	68.6	64.9	57.2
Most polar fraction	3.2	3.6	15.7
Neutral lipids	2.7	2.3	3.8

[a] A mixture of 2-^{14}C-labeled *cis-rac-* (64.7%) and *trans-rac*-2-pentadecyl-4-hydroxymethyl-1,3-dioxolanes (35.3%) was administered.

[b] Identified as unmetabolized substrate by TLC and by GLC of the acetate.

[c] The substrate recovered consisted of 56.0% *cis*-5 and 44.0% *trans*-5 isomers.

incorporated into brain lipids. A mixture of ^{14}C-labeled acetal isomers was injected into the brains of 18-day-old rats, and animals were killed after 6 and 24 hr. The amounts of total radioactivity administered and the activities recovered in total lipids, unmetabolized substrate, polar lipids, and neutral lipids after both time periods are listed in Table 1.

The highest amount of radioactivity from cyclic acetals was found in the polar lipids. A comparison of the distribution of radioactivity in the isomers of the substrate administered and in the unmetabolized substrates recovered after 6 and 24 hr (Table 2) indicated different rates of metabolism for each of the acetal isomers. Relative amounts of radioactivity associated with both of the six-membered ring acetals were increased after elapsed time periods, whereas the radioactivity in the five-membered ring acetals was decreased. The *cis*-5 acetal was most actively metabolized and the *cis*-6 isomer was biologically most inert in this system. It should be mentioned in this context that racemic mixtures of *cis*-5 and *trans*-5 acetals were administered (optical isomers of the six-membered ring acetals do not exist). As preferential enzymatic attack of one of the optical *cis*-5 and *trans*-5 isomers appears most likely, its actual rate of metabolism should be higher than is evident from Table 2. Experimental work towards establishing the diastereospecificity of the system is in progress.

The metabolic behavior of individual *cis*-6 and *trans*-6 cyclic acetal isomers and of a mixture of *cis*-5 and *trans*-5 isomers was studied in animals killed 12 hr after administration of the labeled acetals. Similar amounts of the six-membered ring isomers were injected, whereas the amount of radioactivity injected as five-membered ring isomers was somewhat larger. Table 3 shows the amounts administered and the radioactivities recovered in the total lipids, in unused substrate, in polar lipids, and in neutral lipids. Approximately one-fourth of the radioactivity injected was recovered in the total brain lipids. Only small amounts of radioactivity (2.3–3.8%) were associated with neutral lipids other than unused substrate. As major amounts of activity were found to have been incorporated into polar lipids (28.4–53.4%), aliquots of these were further resolved by TLC (developing solvent, chloroform–methanol–water 65:25:4). In all cases, higher percentages of radioactivity were associated with the PC fractions than with the PE fractions (Table 3). Noteworthy is the relatively high activity (15.7%) of a lipid fraction more polar than PC formed from the *cis/trans*-5 substrate. Its structure is under investigation.

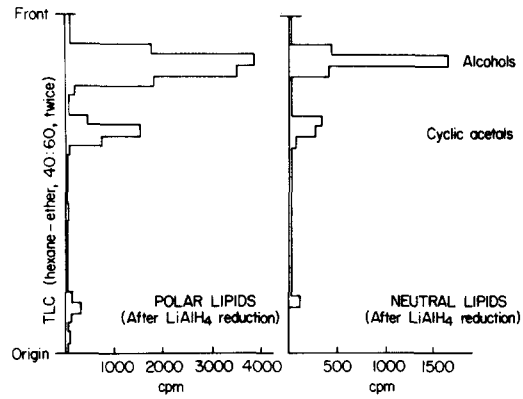


Fig. 2. Distribution of radioactivity among the products of $LiAlH_4$ reduction of polar and neutral rat brain lipids (12 hr after injection of *cis/trans*-5 acetals).

The total polar lipids and the neutral lipids (after removal of unmetabolized substrate) were subjected to lithium aluminum hydride reduction in order to determine whether intact glycerol acetals had been incorporated into the lipids of rat brain, possibly through phosphorylation or acylation of their free hydroxy groups. Hydrogenolysis with lithium aluminum hydride and neutral hydrolysis of the lithium–alumino complex does not affect alkyl or alk-1-enyl ether bonds (27) nor does it cleave or isomerize cyclic acetals (11, 12).

TABLE 4. Distribution of radioactivity among the products of $LiAlH_4$ reduction of polar and neutral lipids (12 hr)

		Cyclic Glycerol Acetal Administered					
		cis-6		*trans*-6		*cis/trans*-5[b]	
Lipid Fraction	R_F Value[a]	PL	NL	PL	NL	PL	NL
Nonpolar constituents	1.0				6.5		1.0
Alcohols[c]	0.64	96.1	97.4	94.0	83.5	78.2	74.7
16:0 (%)		64.9	63.1	68.2	66.7	67.3	66.9
18:0 (%)		25.5	23.6	19.4	20.7	19.8	23.1
18:1 (%)		9.6	13.3	12.4	12.6	12.9	10.0
Cyclic acetals[c]	0.4–0.5	1.0	2.5	1.1	1.2	18.6	20.6
cis (%)						49.8	52.9
trans (%)						50.2	47.1
Alk-1-enyl glycerols[d]	0.26	1.6		0.5	0.5	0.5	0.2
Alkyl glycerols[d]	0.15	1.3	0.1	0.3	0.9	0.5	0.5
Unknown at origin	0			4.1	7.4	2.2	3.0

[a] Fractionation by TLC; developing solvent, hexane–diethyl ether 40:60 (v/v), developed twice.
[b] A mixture of 2-^{14}C-labeled *cis-rac-* (64.7%) and *trans-rac*-2-pentadecyl-4-hydroxymethyl-1,3-dioxolanes (35.3%) was administered.
[c] Identified by TLC and by GLC of their acetates.
[d] Tentatively identified by TLC.

The distribution of radioactivity among the products of lithium aluminum hydride reduction of polar and neutral lipids is shown in Table 4. It is evident, from these data, that when the *cis*-6 and *trans*-6 isomers served as substrates, major amounts of activity were found only in the alcohol fractions, reflecting the conversion of these acetals to the constituent fatty acids of polar and neutral lipids of rat brain. In contrast, with *cis/trans*-5 acetals as substrates, significant amounts of radioactivity were recovered, not only with the long-chain alcohols but with the long-chain cyclic acetals as well (see Fig. 2), demonstrating that the dioxolane isomers had also been incorporated as such into neutral and polar lipids. In all three experiments, only minute quantities of radioactivity were found to be associated with TLC fractions corresponding to alkyl ethers or alk-1-enyl ethers of glycerol (Table 4).

The alcohols derived from the constituent fatty acids of polar and neutral lipids of each experiment were isolated by TLC and analyzed by GLC of their acetates. The distribution of radioactivity among the alkyl acetates derived from the constituent fatty acids was found to be largely independent of the isomer administered and the lipid class (Table 4). Most of the radioactivity was found in the hexadecanoyl groups (63.1–68.2%); less was associated with octadecanoyl (19.4–25.5%) and octadecenoyl moieties (9.6–13.3%).

The cyclic acetals obtained through $LiAlH_4$ reductions of polar and neutral lipid fractions were similarly resolved by GLC of their acetates in the presence of the four unlabeled acetal acetates as carriers. With the *cis*-6 and *trans*-6 isomers as substrates, the radioactivities recovered with the acetal fractions were negligible. When *cis/trans*-5 acetal served as substrate, radioactivity in acetal fractions from polar and neutral lipids was confined to GLC peaks representing the *cis*- and *trans*-dioxolane isomers; no activity was associated with the dioxanes. The data (Table 4) demonstrate complete absence of structural isomerization throughout the experiments, a fact which is also supported by the analyses of the unmetabolized substrates (Table 3).

From the data presented here, one can conclude that long-chain cyclic acetals of glycerol are metabolized in myelinating rat brain largely through a ring-opening mechanism. As the labeling patterns of the constituent acyl groups of polar and neutral lipids closely resemble that previously observed when palmitic acid served as substrate (14), this fatty acid appears to be the major degradation product of C_{16} glycerol acetals. Enzymatic cleavage of the cyclic acetals may proceed via the hemiacetals and subsequent oxidation to hexadecanoic acid, some of which is then elongated and desaturated. The small activities found for alkyl ether and alk-1-enyl ether lipids can satisfactorily be explained by reduction of [1-^{14}C]hexadecanoic acid and incorporation of hexadecanol into ether lipids (14).

The incorporation of the intact cyclic acetals of glycerol into brain lipids, possibly by phosphorylation and acylation, is of significance only for the five-membered ring isomers as substrates. Evidently, the primary hydroxy group of the 1,3-dioxolanes is essential for such reactions to occur.

This investigation was supported in part by Public Health Service research grant no. HL 08214 from the Program Projects Branch, Extramural Programs, National Heart and Lung Institute, by PHS research grants NS 10013, CA 12150, and CA 13113 from the National Institutes of Health, and by the Hormel Foundation.

We also wish to thank Dr. T. Muramatsu, Department of Chemistry, Tokyo Medical and Dental University, for his valuable contributions in the early phases of the project.

Manuscript received 16 April 1973; accepted 28 August 1973.

REFERENCES

1. Anchel, M., and H. Waelsch. 1944. The higher fatty aldehydes. II. Behavior of the aldehydes and their derivatives in the fuchsin test. *J. Biol. Chem.* **152:** 501–509.
2. Pietruszko, R., and G. M. Gray. 1960. Formation of the cyclic acetal phospholipid during alkaline and enzymic hydrolysis of choline plasmalogen. *Biochim. Biophys. Acta.* **44:** 197–199.
3. Pietruszko, R., and G. M. Gray. 1962. The products of mild alkaline and mild acid hydrolysis of plasmalogens. *Biochim. Biophys. Acta.* **56:** 232–239.
4. Davenport, J. B., and R. M. C. Dawson. 1961. Isolation of the cyclic acetal of glycerylphosphorylethanolamine from ox-heart lipids and its probable origin. *Biochem. J.* **79:** 10P–11P.
5. Davenport, J. B., and R. M. C. Dawson. 1962. The formation of cyclic acetals during the acid hydrolysis of lysoplasmalogens. *Biochem. J.* **84:** 490–496.
6. Frosolono, M. F., A. Kisic, and M. M. Rapport. 1967. The structure of plasmalogens. VIII. Preparation and properties of lysophosphatidal choline. *J. Org. Chem.* **32:** 3998–4000.
7. Bergmann, W., and R. A. Landowne. 1958. Contributions to the study of marine products. XLVI. Phospholipids of a sea anemone. *J. Org. Chem.* **23:** 1241–1245.
8. Rapport, M. M., and N. F. Alonzo. 1960. The structure of plasmalogens. V. Lipids of marine invertebrates. *J. Biol. Chem.* **235:** 1953–1956.
9. Ellingboe, J., and M. L. Karnovsky. 1967. Origin of glycerol ethers. Biosynthesis from labeled acetate, stearic acid, stearaldehyde, and stearyl alcohol. *J. Biol. Chem.* **242:** 5693–5699.
10. Wiley, R. A., D. D. Sumner, and E. J. Walaszek. 1970. Structure determinations and synthesis of pharmacologically active phospholipids from kidney and intestine. *Lipids.* **5:** 803–811.
11. Baumann, W. J. 1971. Configuration and conformation of the long-chain cyclic acetals of glycerol. *J. Org. Chem.* **36:** 2743–2747.
12. Baumann, W. J., T. H. Madson, and B. J. Weseman. 1972. "Plasmalogen-type" cyclic acetals: formation and conformation of the 1,3-dioxanes and 1,3-dioxolanes from 1-*O*-*cis*-alk-1'-enyl-*sn*-glycerols. *J. Lipid Res.* **13:** 640–650.

13. Korey, S. R., and M. Orchen. 1959. Plasmalogens of the nervous system. I. Deposition in developing rat brain and incorporation of C^{14} isotope from acetate and palmitate into the α,β-unsaturated ether chain. *Arch. Biochem. Biophys.* **83:** 381–389.
14. Schmid, H. H. O., and T. Takahashi. 1970. Reductive and oxidative biosynthesis of plasmalogens in myelinating brain. *J. Lipid Res.* **11:** 412–419.
15. Muramatsu, T., and H. H. O. Schmid. 1971. Formation of 1-*O*-2′-hydroxyalkyl glycerophosphatides from 1,2-heptadecanediol in myelinating brain. *J. Lipid Res.* **12:** 740–746.
16. Muramatsu, T., and H. H. O. Schmid. 1972. On the formation of 1-*O*-2′-hydroxyalk-1′-enyl or 1-*O*-2′-ketoalkyl glycerophosphatides from 1,2-alkanediol in myelinating brain. *Biochim. Biophys. Acta.* **260:** 365–368.
17. Su, K. L., and H. H. O. Schmid. 1972. Metabolism of long-chain polyunsaturated alcohols in myelinating brain. *J. Lipid Res.* **13:** 452–457.
18. Su, K. L., and H. H. O. Schmid. 1972. On the oxidation of long-chain polyunsaturated alcohols by myelinating rat brain. *Biochem. Biophys. Res. Commun.* **48:** 94–100.
19. Su, K. L., and H. H. O. Schmid. 1972. Metabolism of *n*-hexadecane in myelinating rat brain. *FEBS Lett.* **25:** 316–318.
20. Schmid, H. H. O., T. Muramatsu, and K. L. Su. 1972. On the nonconversion of alkyl acyl choline phosphatides to the corresponding plasmalogens in myelinating rat brain. *Biochim. Biophys. Acta.* **270:** 317–323.
21. Muramatsu, T., and H. H. O. Schmid. 1973. Metabolism of 1-hydroxy-2-ketoheptadecane in myelinating brain. *Biochim. Biophys. Acta.* **296:** 265–270.
22. Chang, N., T. Muramatsu, and H. H. O. Schmid. 1973. Structural specificity in the formation of glycerol ethers and plasmalogens in myelinating brain. *Biochim. Biophys. Acta.* **306:** 437–445.
23. Schlenk, H., and J. L. Gellerman. 1960. Esterification of fatty acids with diazomethane on a small scale. *Anal. Chem.* **32:** 1412–1414.
24. Brown, W. G. 1951. Reductions by lithium aluminum hydride. *Org. React.* **6:** 469–509.
25. Baumann, W. J., and H. K. Mangold. 1964. Reactions of aliphatic methanesulfonates. I. Syntheses of long-chain glyceryl-(1) ethers. *J. Org. Chem.* **29:** 3055–3057.
26. Mahadevan, V., F. Phillips, and W. O. Lundberg. 1966. Reactions of dimethyl sulfoxide with sulfonate esters of fatty alcohols. I. Synthesis of higher saturated and unsaturated fatty aldehydes. *Lipids.* **1:** 183–187.
27. Schmid, H. H. O., W. J. Baumann, and H. K. Mangold. 1967. Alkoxylipids. III. Naturally occurring D(+)-1-*O*-*cis*-alk-1′-enyl diglycerides. *Biochim. Biophys. Acta.* **144:** 344–354.