PREPARATION AND PROPERTIES OF OPTICALLY ACTIVE DERIVATIVES OF GLYCEROL[1]

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I. INTRODUCTION

Asymmetric substitution in the glycerol molecule produces compounds which are capable of occurring in enantiomorphic forms. This is the case with all α-monoglycerides and α,β-diglycerides as well as α,α′-diglycerides containing unlike substituents and triglycerides in which the α- and α′-positions are substituted by different acyl residues.

This type of asymmetry has been observed in nature in only a few instances. The optically active α-glycerophosphoric acid and the lecithins and cephalins related to it have long been known, while the optically active glycerol ethers such as batyl alcohol have only recently been isolated from natural sources. It might be predicted, however, that the occurrence of glycerides in nature as enantiomorphs is much more widespread than experimental evidence up to the present time indicates. This may be explained by the observation that the optical rotation of stereoisomeric

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triglycerides containing higher fatty acid residues is not detectable. Since the bulk of natural fats is composed of triglycerides, and since nature strongly favors the elaboration of mixed acid and not "simple" triglycerides, it might be expected that this fact has considerable significance. It is conceivable, for instance, that enzymes (e.g., lipase) in their action on the enantiomorphic glycerides might distinguish between the two isomers in an analogous manner to the distinction shown in the case of sugars and amino acids.

The possibility of isolating a pure, optically active individual glyceride from a natural source seems at present remote, since the methods generally employed are such that asymmetry, if present, might be easily destroyed during the process of isolation (acyl migration). The alternative approach to these interesting compounds is to find a reliable means for their synthesis.

A number of methods based on two entirely different principles are available up to the present for the synthesis of optically active glycerides. The first of these general principles requires resolution of intermediate compounds, which then can be converted into the corresponding glycerides. This naturally involves the general difficulty of eliminating, without racemization, the acid or basic residues originally introduced to make possible the resolution. The alternative possibility is to start the synthesis with an optically active natural compound which can be converted without racemization to the desired glycerides. The former principle, involving resolution, has received considerable attention in the laboratories of Abderhalden, Bergmann, and Grün; the second principle has been utilized only recently (7, 8, 9, 10).

Abderhalden and Eichwald (1) resolved racemic aminodibromopropane by means of *d*-tartaric acid. The optical antipodes so obtained were employed to prepare optically active α-monoglycerides and α,β-diglycerides (2), as shown in chart I.

Abderhalden and Eichwald assigned the prefix *d*- or *l*- to these compounds, according to the direction of the observed rotation.

Bergmann and Sabetay (13) resolved the α-acyl esters of γ-aminopropylene glycol by means of saccharic acid, and the resulting enantiomorphs were then treated with nitrous acid to produce α-monoglycerides. Similarly, Bergmann, Brand, and Dreyer (14) converted α,β-diacyl esters of γ-aminopropylene glycol into α,β-diglycerides.

In contrast to the method of Abderhalden and Bergmann, both of whom introduced the basic amino group into the glycerol molecule, Grün and Limpächer (27) attempted the resolution of the glycerides by fractional crystallization of the strychnine salts of their sulfuric acid esters. The potassium salts of the resulting sulfuric acid esters of the glycerides showed extremely high rotations in benzene solutions, the rotations being, how-

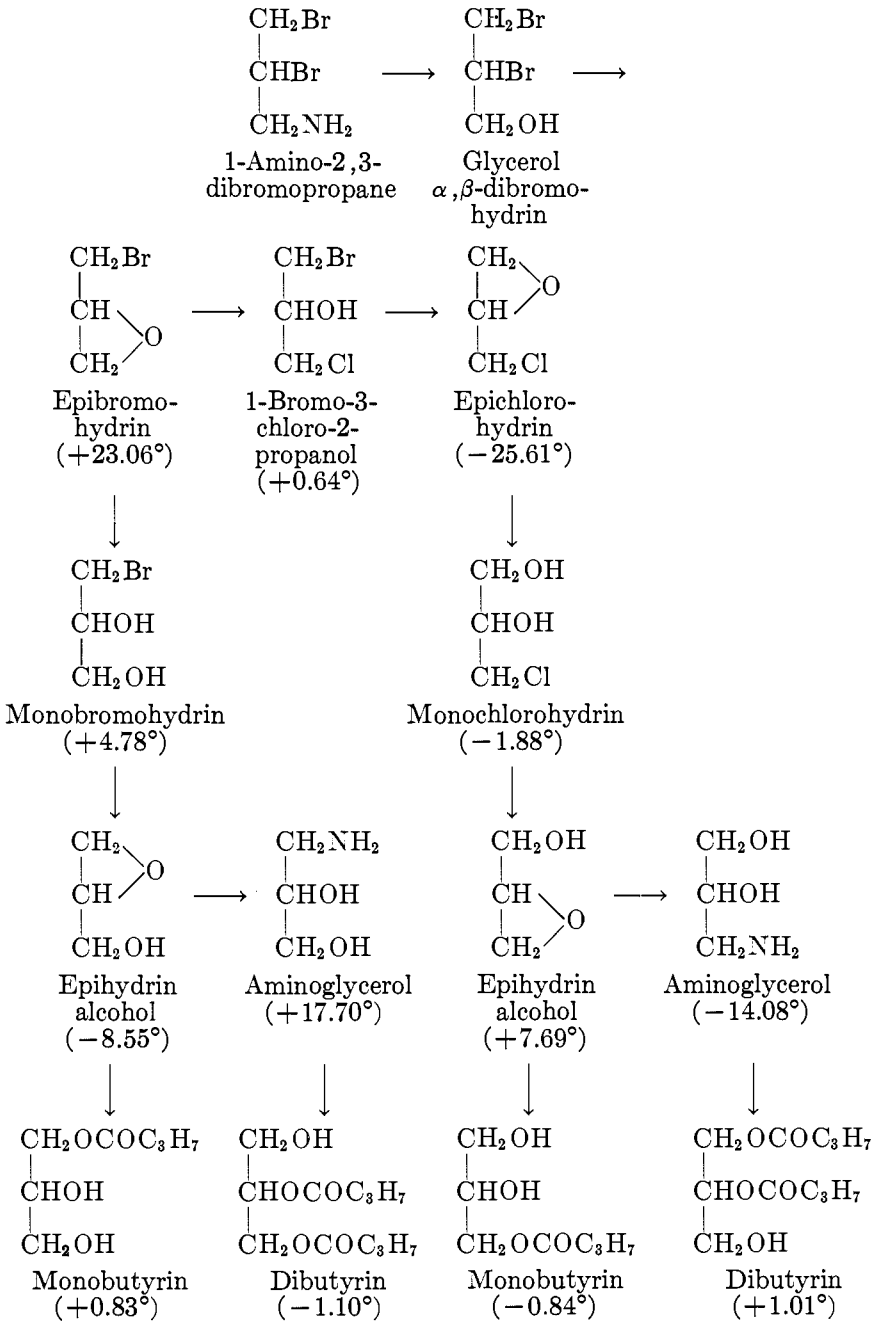
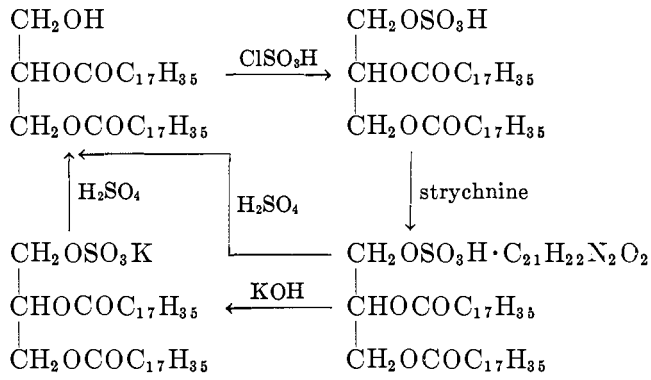

CH2Br
CHBr
CH2NH2
1-Amino-2,3-dibromopropane
CH2Br
CHBr
CH2OH
Glycerol α,β-dibromohydrin
CH2Br
CH
O
CH2
Epibromohydrin (+23.06°)
CH2Br
CHOH
CH2Cl
1-Bromo-3-chloro-2-propanol (+0.64°)
CH2
O
CH
CH2Cl
Epichlorohydrin (−25.61°)
CH2Br
CHOH
CH2OH
Monobromohydrin (+4.78°)
CH2OH
CHOH
CH2Cl
Monochlorohydrin (−1.88°)
CH2
O
CH
CH2OH
Epihydrin alcohol (−8.55°)
CH2NH2
CHOH
CH2OH
Aminoglycerol (+17.70°)
CH2OH
CH
O
CH2
Epihydrin alcohol (+7.69°)
CH2OH
CHOH
CH2NH2
Aminoglycerol (−14.08°)
CH2OCOC3H7
CHOH
CH2OH
Monobutyrin (+0.83°)
CH2OH
CHOCOC3H7
CH2OCOC3H7
Dibutyrin (−1.10°)
CH2OH
CHOH
CH2OCOC3H7
Monobutyrin (−0.84°)
CH2OCOC3H7
CHOCOC3H7
CH2OH
Dibutyrin (+1.01°)

CHART I

ever, dependent on temperature to a surprising degree.[2] The acid hydrolysis of these sulfuric acid compounds yielded products with no detectable rotation. The reactions as applied to α,β-distearin are represented in the following diagram:



Esters of glycerol with polybasic organic acids are also resolvable as salts of active bases. For example, Suzuki and Inoue (47) resolved the

TABLE 1

Properties of glycerides and intermediates used in the synthesis of glycerides

COMPOUND	OPTICAL ROTATION OF PRODUCT OBTAINED	
	By resolution	From acetoneglycerol
α-Monobutyrin	±0.84°*	±2.2°
$d(-)$-α,β-Distearin	0°†	−2.7° (in $CHCl_3$)
α-(p-Nitrobenzoyl)-α',β-dibenzoylglycerol	−2.1° (in $C_2H_2Cl_4$)‡	−19.9° (in $C_2H_2Cl_4$)
$l(+)$-Epihydrin alcohol	+7.69°*	+15°
γ-Aminopropylene glycol (in HCl solution)	−14.08°*	−29°

* Abderhalden and Eichwald.
† Grün and Limpächer.
‡ Bergmann and Sabetay.

half-ester of phthalic acid with α,β-dibenzoylglycerol by means of strychnine into the stereoisomeric forms which were optically active.

The second general principle available for the synthesis of optically active glycerides, as mentioned previously, makes use of optical activity already present in a natural product capable of being converted without

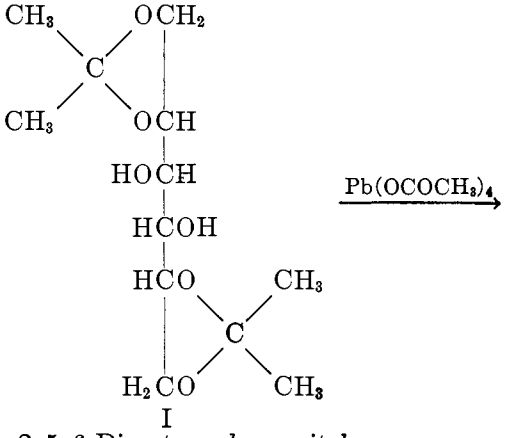
[2] A similar dependence of rotation on temperature has been observed with certain phosphatides and phosphatidic acids (27, 41, 43).

racemization to the desired glyceride. This might be compared with the transformation of carbohydrates into optically active fats, which nature probably carries out by enzymatic reactions in plants and in animal bodies. This principle has been employed by the authors during the last few years and has led to the preparation of a number of active glycerides. The method involves the use of *d*- and *l*-mannitol, from which, by appropriate reactions, the enantiomorphic *d*(+)- and *l*(−)-acetoneglycerols can be obtained (6, 7). Acylation of these compounds according to the method of Fischer, Bergmann, and Bärwind (24), which will be described later, produced the various enantiomorphic glycerides. In all instances observed, this principle of using an inherent natural activity appeared to be preferable to any method involving the principle of resolution, since the glycerides and intermediates invariably possessed greater optical activity than those in corresponding compounds prepared by resolution. Some data on glycerides and related compounds used as intermediates for glyceride synthesis, prepared by both methods, are compared in table 1.

II. PREPARATION OF THE ENANTIOMORPHIC ACETONEGLYCEROLS

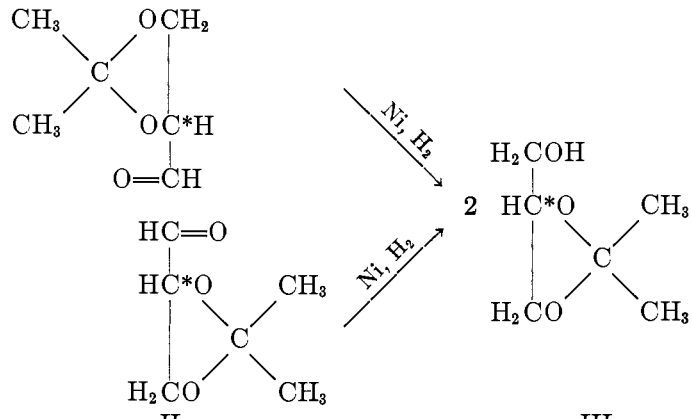
1,2,5,6-Diacetone-*d*-mannitol (I) was prepared by the action of acetone and zinc chloride on *d*-mannitol. The carbon chain of the diacetone-*d*-mannitol was split by lead tetraacetate, with the result that two molecules of acetonated *d*-glyceraldehyde (II) (7) were formed. This compound was reduced catalytically in ethyl acetate solution with hydrogen under pressure, using the Raney nickel catalyst or the nickel catalyst according to Rupe (42), and yielded *d*(+)-acetoneglycerol (III) in an excellent yield.

Starting with *l*-mannitol, exactly the same procedure was followed to produce *l*(−)-acetoneglycerol. However, unlike *d*-mannitol, which is a



I

1,2,5,6-Diacetone-*d*-mannitol



II
Acetone-*d*-glyceraldehyde

III
d(+)-Acetoneglycerol

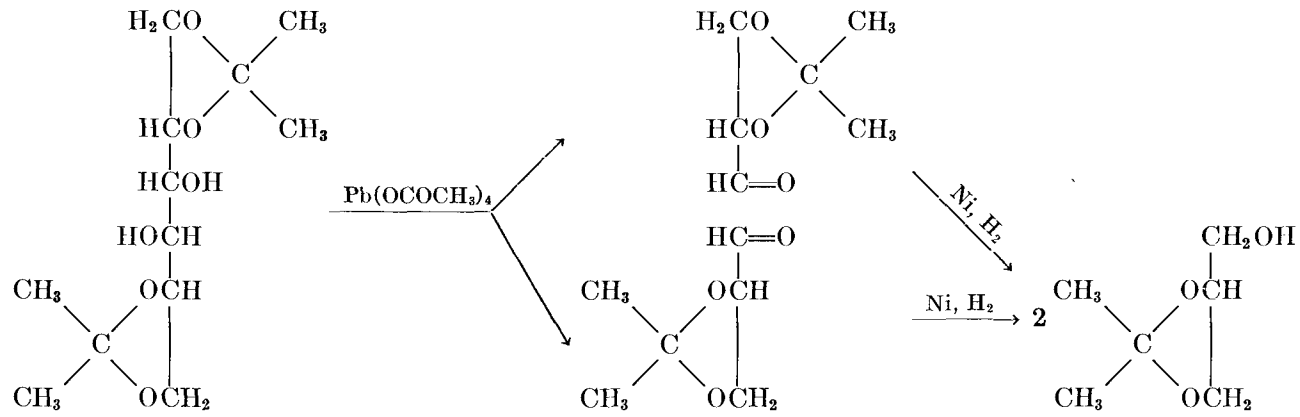
commercially available substance, *l*-mannitol must be prepared by a tedious series of reactions starting from *l*-arabinose. The *l*-arabinose itself is obtained from Arizona mesquite gum by the method of Anderson and Sands (4). The arabinose is then transformed by cyanohydrin synthesis into *l*-mannonolactone (33), and the latter is reduced catalytically in one operation to *l*-mannitol (20). Cleavage of the 1,2,5,6-diacetone-*l*-mannitol (IV) with lead tetraacetate then produces two molecules of acetone-*l*-glyceraldehyde (V). The reduction of this compound with nickel and hydrogen leads to the formation of *l*(−)-acetoneglycerol (VI).

The rotations of the two enantiomorphic acetoneglycerols prepared in this way were found to be of equal magnitude ($[\alpha]_D = \pm 12.6°$). Since the acetoneglycerols were prepared from 1,2,5,6-diacetonemannitols of known structure (15), their constitution as 1,2-acetoneglycerols was also established. As an additional proof of structural uniformity, the *d*(+)-acetoneglycerol was converted by methylation and hydrolysis to glycerol α-monomethyl ether. The latter, on titration with periodic acid (7), proved to be pure glycerol α-methyl ether. The optical rotation of *d*(+)-acetoneglycerol was unaltered by successive benzoylation, purification of the high-boiling benzoate, and saponification to the original, optically active acetoneglycerol.

It should be pointed out that the asymmetry of the acetone-*d*-glyceraldehyde is maintained in the glycerol series only by the initial presence of the isopropylidene group in the asymmetrical 1,2-position. Should the molecule become symmetrical at any stage in the operations, a subsequent asymmetrical substitution would yield only a racemic compound.

By an appropriate series of reactions in which the asymmetry is maintained throughout, *d*(+)-acetoneglycerol can be transformed into *l*(−)-acetoneglycerol (25). The reactions shown in chart II are involved.

l-Arabinose ⟶ *l*-Mannonolactone ⟶ *l*-Mannitol ⟶



IV
1,2,5,6-Diacetone-*l*-mannitol

V
Acetone-*l*-glyceraldehyde

VI
l(−)-Acetoneglycerol

The interconversion of optical antipodes is reminiscent of the work of Emil Fischer and Brauns (22) on the *d*- and *l*-isopropylmalonamic acids, in which the asymmetry was maintained by substituents on the two carboxyl groups.

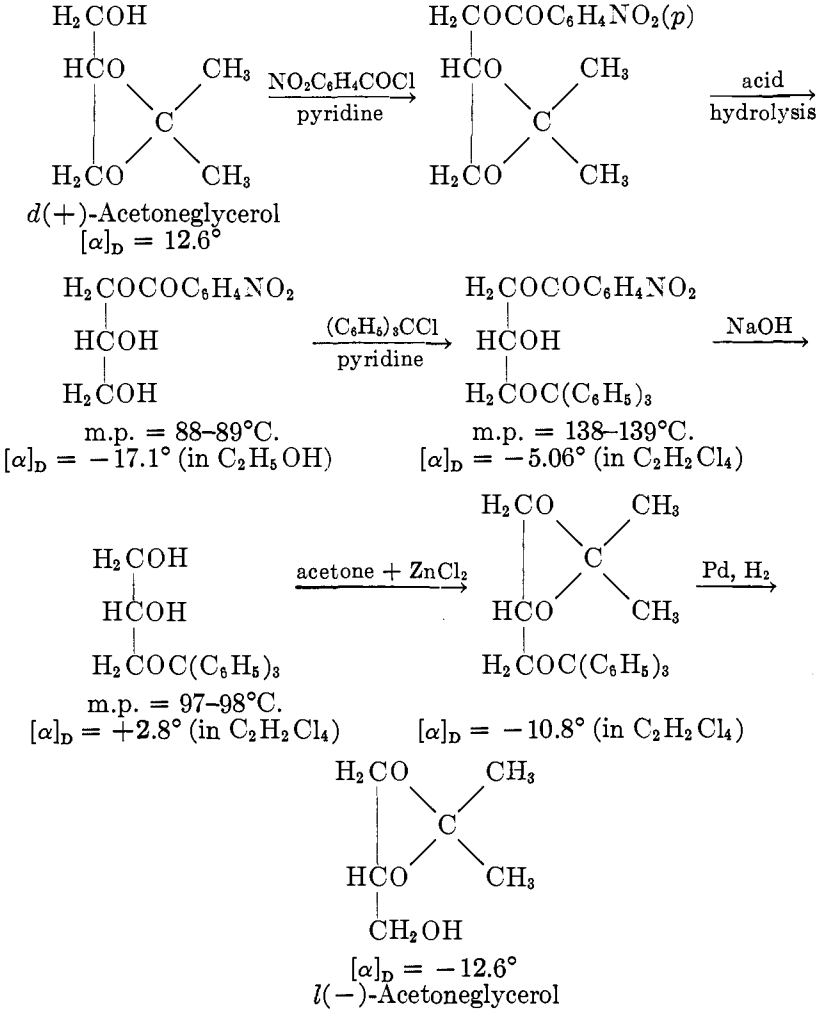


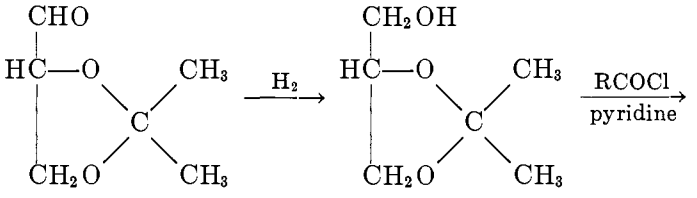
CHART II

III. PREPARATION OF GLYCERIDES

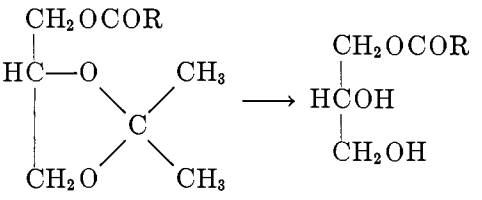
A. α-Monoglycerides and triglycerides

Possession of the *d*(+)- and *l*(−)-acetoneglycerols opened the way for the preparation of optically active glycerides, according to the method of

synthesis used by Emil Fischer, Bergmann, and Bärwind (24) for the corresponding racemic compounds. This method consists in introducing the acyl group into the free hydroxyl position by the action of acid chloride and quinoline or pyridine. Mild acid hydrolysis then removes the protecting acetone group, yielding an optically active α-monoglyceride. The synthesis of an α-monoglyceride from acetone-*d*-glyceraldehyde is represented by the following equations:



Acetone-*d*-glyceraldehyde



l(−)-α-Monoglyceride

The acetonated α-monoglycerides listed in table 2 have been prepared from *d*(+)-acetoneglycerol.[3]

By acid hydrolysis of the corresponding acetone compounds, the optically active α-monoglycerides listed in table 3 have been obtained.

Since there is a certain danger of racemization, due to acyl migration, during the acid hydrolysis of the protecting acetone group, a number of experiments were conducted to ascertain the purity of the enantiomorphic α-monoglycerides. Titration of the 1,2-glycol content with lead tetraacetate by the methods of Criegee (16) and Malaprade (38) showed the products to be pure α-monoglycerides. This evidence, however, does not eliminate the possibility that racemization may have occurred during the acid treatment. Only if re-acetonation of the monoglyceride results in an α-acyl-α',β-acetoneglycerol of the correct rotation can it be concluded that the free monoglyceride is a pure enantiomorph.

The ease of removal of the acetone residue by means of acid hydrolysis varies considerably with the nature of the acyl residue in the acetonated

[3] Note the change of nomenclature from that used in reference 8. The acetone compounds are now given the same steric classification as the monoglycerides prepared from them.

TABLE 2

Acetonated l-α-monoglycerides

ACETONE COMPOUND OF	BOILING POINT	MELTING POINT	REFRACTIVE INDEX	ROTATION	SPECIFIC ROTATION	SOLVENT
	°C.	°C.				
l(−)-α-Acetylglycerol	85–86 at 10–11 mm.		$n_D^{15°} = 1.4288$		$[\alpha]_D = +3.24°$	Homogeneous substance
l(−)-α-Propionylglycerol	88–89 at 7 mm.		$n_D^{20°} = 1.4260$		$[\alpha]_D = +3.6°$	Homogeneous substance
l(−)-α-Butyrylglycerol	97–98.5 at 7 mm.		$n_D^{25°} = 1.4270$		$[\alpha]_D = +4.92°$	Homogeneous substance
l(−)-α-Caproylglycerol	118–120 at 7 mm.		$n_D^{25.5°} = 1.4322$		$[\alpha]_D = +4.5°$	Homogeneous substance
l(−)-α-Laurylglycerol	130–131 at 0.002 mm.		$n_D^{20°} = 1.4448$		$[\alpha]_D^{21°} = +3.42°$	Homogeneous substance
l(−)-α-Stearylglycerol		43.5		$\alpha_D^{50°} = +3.0$ to $+3.5$		Fused substance, 1 dm. tube
					$[\alpha]_D = +1.9°$	Pyridine
l(−)-α-Palmitylglycerol		33–35		$\alpha_D = +4.38°$		Fused substance, 1 dm. tube
					$[\alpha]_D = +2.48°$	Pyridine

α-monoglyceride, and consequently the conditions of the hydrolysis must be adapted in each instance to the specific compound in hand.

TABLE 3

l-α-Monoglycerides

COMPOUND	MELTING POINT	SPECIFIC ROTATION	SOLVENT
	°C.		
l(−)-α-Butyrylglycerol (liquid)		$[\alpha]_D = -6.0°$	Pyridine
		$[\alpha]_D = -2.2°$	Homogeneous substance
l(−)-α-Laurylglycerol	53–54	$[\alpha]_D = -3.76°$	Pyridine
l(−)-α-Stearylglycerol	76–77	$[\alpha]_D = -3.58°$	Pyridine
l(−)-α-Palmitylglycerol	71–72	$[\alpha]_D = -4.37°$	Pyridine
l(−)-α-(*p*-Toluenesulfonyl)glycerol	63–64	$[\alpha]_D = -7.3°$	Pyridine
l(−)-α-(*p*-Nitrobenzoyl)glycerol	88–89	$[\alpha]_D = -17.1°$	Ethanol

TABLE 4

Acetone compounds of d-α-monoglycerides; d(+)-α-butyrylglycerol

SUBSTANCE	BOILING POINT	REFRACTIVE INDEX	SPECIFIC ROTATION	SOLVENT
	°C.			
Acetone compound of:				
d(+)-α-Propionylglycerol	88–89 at 7 mm.	$n_D^{24°} = 1.4269$	$[\alpha]_D = -3.6°$	Homogeneous substance
d(+)-α-Butyrylglycerol	97–99 at 7 mm.	$n_D^{23°} = 1.4280$	$[\alpha]_D = -4.9°$	Homogeneous substance
d(+)-α-Caproylglycerol	118–120 at 7 mm.	$n_D^{20°} = 1.4349$	$[\alpha]_D = -4.5°$	Homogeneous substance
Free α-monoglyceride:				
d(+)-α-Butyrylglycerol			$[\alpha]_D = +2.2°$	Homogeneous substance

From the difficultly available *l*(−)-acetoneglycerol were prepared the three acetone compounds of α-monoglycerides and one free α-monoglyceride listed in table 4.

In the active α-monoglycerides containing aromatic substituents, the rotations remain unchanged after standing for a period of one year, indicating that the acyl residues do not undergo migration. This is in agreement with the findings of Jackson and King (29), who observed no acyl migration in glycerides containing aromatic acid radicals. The α-monoglycerides derived from aliphatic acids, however, show a gradual decrease of rotation even in the crystalline state at room temperature, amounting to one-half to one-third of the original rotation during one year (9).

In view of this instability of the pure monoglycerides *in vitro*, one is led to speculate on the possibility of enantiomorphic glycerides existing and retaining their steric configuration *in vivo*. The recent work of Schoenheimer (44) has brought forth new evidence that the fats involved in metabolism in the animal body undergo rapid and constant chemical change. Any such lability in the case of the glycerides taking part in fat metabolism would appear at first sight to make impossible the existence of any stable stereoisomerism. However, it is conceivable that the interchange of acyl residues of the glycerides *in vivo* would not necessarily result in racemization, but rather that the configuration of the glycerol part of the molecule would be retained during these chemical changes by the specific action of the enzymes, and that this configuration would comprise, indeed, one of the stable characteristics of the cell.

From the α-monoglycerides, triglycerides containing different acyl residues can be obtained by further treatment with acid chlorides and quinoline or pyridine. To eliminate the possibility that the original materials—the active α-monoglycerides—had become racemized by migration of the acyl group under the influence of the tertiary base (pyridine or quinoline) used in the above preparations, the monoglycerides were exposed for some time to the action of pyridine. There was, however, no change in rotation observable, and the glycerides, when recovered from the solvent, were shown by titration by the method of Criegee to be pure α-monoglycerides. From *l*(−)-α-lauryl-, *l*(−)-α-stearyl-, *l*(−)-α-palmityl-, and *l*(−)-α-(*p*-nitrobenzoyl)-glycerol, respectively, the triglycerides listed in table 5 were prepared.

In spite of the distinct rotation shown by the monoglycerides, most triglycerides prepared from them show no rotation whatever in the solvents used, either in sodium light or in ultraviolet light. This possession of asymmetry without measurable optical activity may well be a characteristic of triglycerides with fatty acid residues, because α-(*p*-nitrobenzoyl)-α,β-dibenzoylglycerol possesses the considerable rotation of $[\alpha]_D = -19.9°$.

The lack of observable rotation in the synthetic aliphatic unsymmetrical triglycerides with at least two different long-chain fatty acid residues leads to the suspicion that the *natural* triglycerides of the same type may also

show no rotation. Therefore, natural asymmetric triglycerides, though they do not show a rotation, are not necessarily racemic, but might easily occur in either of the two possible enantiomorphic forms.

Since, in contrast to the triglycerides, the α-monoglycerides show a relatively large rotation, it is possible that the slight and gradually disappearing rotation sometimes observed (46, 47) in freshly isolated natural fats and oils is due to the presence of α-monoglycerides, especially since, as mentioned before, synthetic α-monoglycerides show a gradual decrease in rotatory power on standing at room temperature.

B. Steric classification and nomenclature of glycerides

With regard to the question of steric relationship, the optical classification of the enantiomorphic α-monoglycerides is established comprehensively by relating them to the corresponding *d*- and *l*-glyceraldehydes.

TABLE 5

Synthetic triglycerides prepared from α-monoglycerides

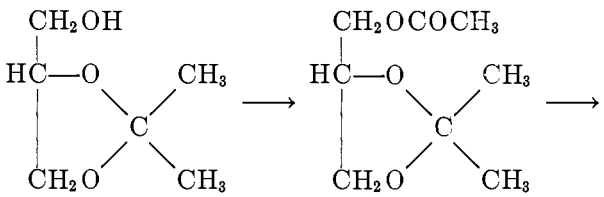
TRIGLYCERIDE	MELTING POINT	α_D
	°C.	
α-Lauryl-α′,β-distearylglycerol	48.5	0.0° (in pyridine)
α-Stearyl-α′,β-dipalmitylglycerol	62.5	0.0° (in chloroform or pyridine)
α-Palmityl-α′,β-dilaurylglycerol	44	0.0° (in pyridine)
α-(*p*-Nitrobenzoyl)-α′,β-distearylglycerol	67–67.5	−1.4° (in chloroform)
α-(*p*-Nitrobenzoyl)-α′,β-dibenzoylglycerol	87–88	−19.9° (in tetrachloroethane)

This concept has a precedent in the classification of the sugars according to Wohl and Freudenberg (52). Thus an α-monoglyceride which would be formed by the reduction of a 3-acyl-*d*-glyceraldehyde is called a *d*-α-monoglyceride, while a glyceride originating from a 3-acyl-*l*-glyceraldehyde is an *l*-α-monoglyceride. In other words, an α-monoglyceride is to be put in the same category with that glyceraldehyde into which it could be transformed by oxidation without any alteration or removal of substituents. This opinion is directly opposed to that of Abderhalden and Eichwald (1), who hold the view that *no* steric classification of the α-monoglycerides is possible, since members of either series may be obtained from the same original material.

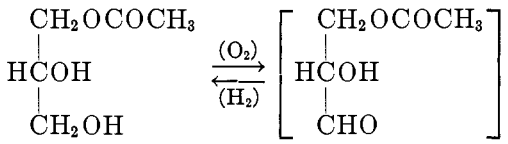
In the preparation of the α-monoglycerides from acetone-*d*-glyceraldehyde through *d*(+)-acetoneglycerol by acylation of the newly formed hydroxyl group and splitting off the acetone, the new substituent has been introduced into the hydroxyl group of that carbon atom which was part

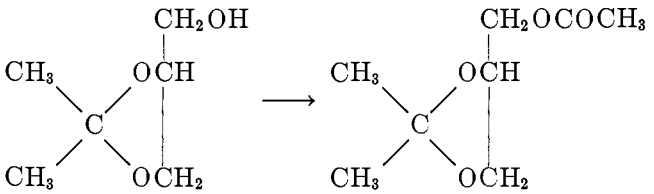
of the carbonyl group of the original acetone-*d*-glyceraldehyde. The α-monoglycerides prepared from *d*(+)-acetoneglycerol therefore belong to the *l*-glyceraldehyde series and are to be designated *l*-glycerides, for if one were to oxidize the α-monoglycerides thus obtained, the result, according to the assumptions discussed above, would be 3-acyl-*l*-glyceraldehydes. Therefore a change of configuration has taken place, and we find ourselves faced with the remarkable fact that application of the sequence of reactions mentioned above to a derivative of *d*-glyceraldehyde yields a derivative of *l*-glyceraldehyde. This transformation from one steric series into the other one is made possible only by the peculiar kind of asymmetry in the glycerol series, an asymmetry which depends entirely on *substitution*.

The way from *d*(+)-acetoneglycerol to an acyl derivative of *l*-glyceraldehyde, and from *l*(−)-acetoneglycerol to a derivative of *d*-glyceraldehyde, is demonstrated below:

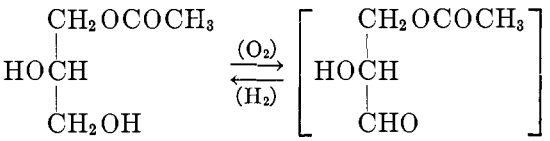


d(+)-Acetoneglycerol





l(−)-Acetoneglycerol



This reasoning can be extended without constraint to the α,β-diglycerides, the α-glycerophosphoric acids, and the α-glycerol ethers. In these

classes of compounds the α-position occupied by the free hydroxyl group is considered to be the potential carbonyl group of that glyceraldehyde to which the particular compound is to be related.

In the case of mixed acid triglycerides this steric classification is arbitrary but may be applied if a chemical or biological consideration gives preferred importance to one of the α-substituents. To illustrate this viewpoint the following example may be cited:

Consider an α-phosphoryl α',β-distearin arising from $l(-)$-glycerophosphoric acid by the introduction of two stearyl residues. If this triglyceride is to be subjected to phosphatase action, its relation to the original $l(-)$-glycerophosphoric acid would govern the course of the hydrolysis, and consequently the relationship to l-glyceraldehyde should be maintained and expressed in the nomenclature. On the other hand, if the same triglyceride is to be subjected to the action of lipase, the stearyl groups are primarily affected, and the compound would be classified as a derivative of d-α,β-distearin and hence related to d-glyceraldehyde.

C. α,β-Diglycerides[4]

Optically active $d(+)$-acetoneglycerol has also been used to prepare what is believed to be the first example of an optically pure enantiomorph of an α,β-diglyceride, namely $d(-)$-α,β-distearin. Attempts to prepare this type of optically active glyceride, as previously indicated in the introduction, have been based on the principle of resolution of racemic intermediate compounds. Abderhalden and Eichwald (2) prepared the enantiomorphic epihydrin alcohols through resolution, and converted these to the corresponding enantiomorphic α-aminopropylene glycols. The amino alcohols were then converted to optically active α,β-diglycerides by the introduction of two O-acyl groups, followed by elimination of the amino group with nitrous acid. In a somewhat similar way, Bergmann, Brand, and Dreyer (14) resolved the di-O-benzoate of α-aminopropylene glycol. Elimination of the amino group with nitrous acid produced an α,β-dibenzoin which was converted to an optically impure p-nitrobenzoate.

By a series of reactions starting with $d(+)$-acetoneglycerol, it has recently been demonstrated[4] that the epihydrin alcohols and α-aminopropylene glycols used by Abderhalden and Eichwald were not optically pure,

[4] The section on α,β-diglycerides was written by John C. Sowden and is based on experimental work carried out by him in this laboratory. The publications of John C. Sowden and H. O. L. Fischer pertaining to the α,β-diglycerides and epihydrin alcohol will appear in the near future, as communications X and XI in the series of "Studies on Acetoneglyceraldehyde and Optically Active Glycerides" by H. O. L. Fischer and Erich Baer.

and thus the enantiomorphic diglycerides described by them must also have been optically impure.

The reactions involved in the preparation (and proof of optical purity) of *l*(+)-epihydrin alcohol[5] and *l*(−)-α-aminopropylene glycol[5] are represented in the scheme in chart III.

It appears safe to assume that the steps involved in scheme A are such that no racemization can occur. Thus the reactions in scheme B must also have proceeded without racemization, and the *l*(+)-epihydrin alcohol

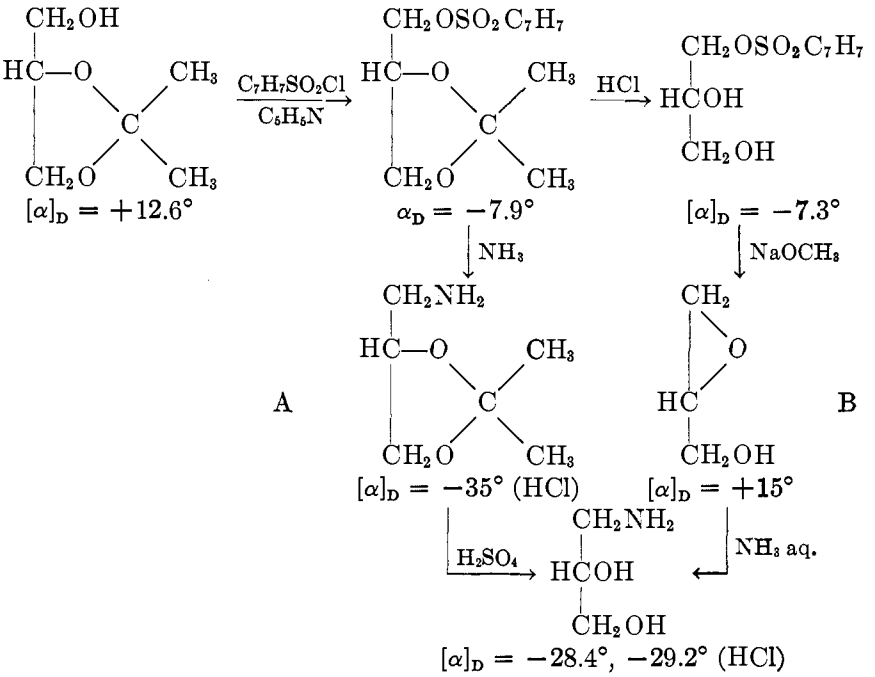


CHART III

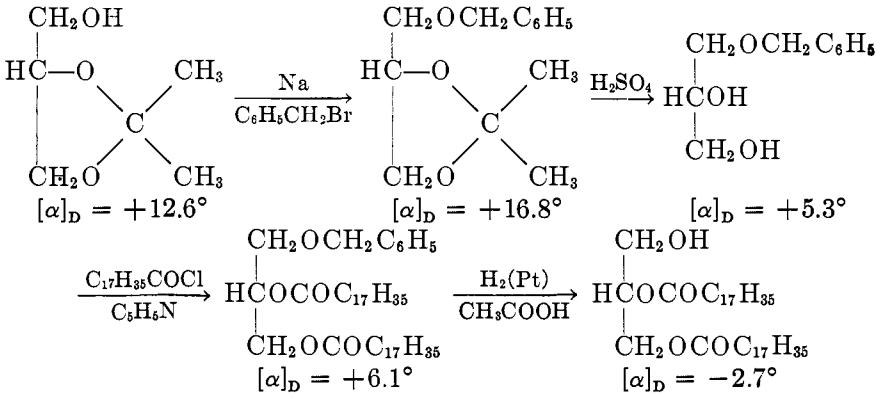
and *l*(−)-γ-aminopropylene glycol are optically pure. Apparently no measurable amount of 1,3-oxide is produced in the formation of the epihydrin alcohol from *l*(−)-α-tosylglycerol, since this isomeric oxide would produce racemic γ-aminopropylene glycol in the next step, and result in a lowering of the optical activity.

In agreement with the observation of L. Knorr and E. Knorr (36), who worked with racemic epihydrin alcohol, the addition of ammonia to *l*(+)-epihydrin alcohol apparently yields exclusively the α-amine. Moreover,

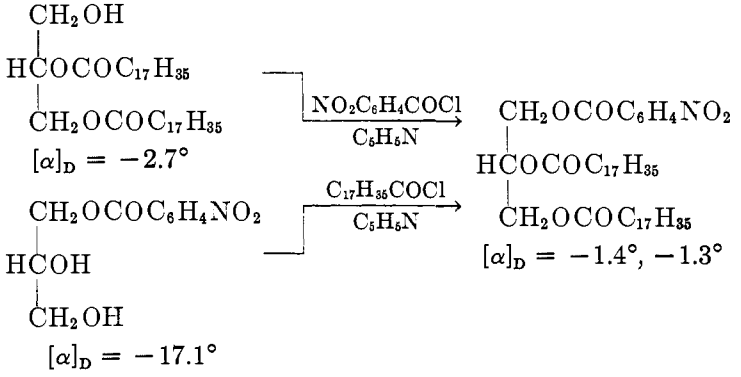
[5] The prefix "*l*" is assigned here by considering the epihydrin alcohol as related to the oxide of *l*-glyceraldehyde and the γ-aminopropylene glycol as related to an *l*-glyceraldehyde in which the hydroxyl on carbon atom 3 is replaced by the amino group.

the addition seems to proceed without Walden inversion, since the resulting amine has the same configuration as that obtained from α-tosyl-$d(+)$-acetoneglycerol. This latter observation is interesting in the light of the results of Levene and Walti (37), who observed that the degree of Walden inversion which occurs during the addition of water to optically active propylene glycol depends largely on the pH of the solution.

For the preparation of optically pure $d(-)$-α,β-distearin from $d(+)$-acetoneglycerol, the following reactions were employed:



As proof of the optical purity of the diglyceride, it was converted to the *p*-nitrobenzoate and compared with the product from the known $l(-)$-α-(*p*-nitrobenzoyl)glycerol (25) and stearyl chloride.



It is a necessary condition that the α,β-diglyceride containing aliphatic ester groups shall at no time be subjected to the effect of mineral acids, since these have been demonstrated to cause acyl migration (21, 29). The benzyl ether was chosen for the diglyceride synthesis because it is stable to acid and alkali but can be removed readily by hydrogenation.

The benzyl group was introduced by the reaction of benzyl bromide with the sodium salt of $d(+)$-acetoneglycerol, the latter being prepared

either with metallic sodium in anhydrous ethyl ether or with sodium naphthalene (45, 51) in dimethyl glycol ether. In order to demonstrate that the reaction with sodium caused no racemization in the acetoneglycerol molecule, the sodium salt was also used through reaction with methyl iodide, to prepare the known methyl ether of $d(+)$-acetoneglycerol, $[\alpha]_D = +22.5°$ (7).

In the removal of the benzyl group from the distearin benzyl ether with platinic oxide (Adams catalyst) and hydrogen, it was found that no hydrogenation occurred when ethyl acetate was used as solvent. However, hydrogenation proceeded smoothly in glacial acetic acid at room temperature. Acetic acid in the cold apparently caused no migration of the acyl groups, as evidenced by the proof of asymmetry through the distearin *p*-nitrobenzoate.

The optically active distearin was also converted to the acetate, but the resulting triglyceride showed no perceptible optical rotation. This is in agreement with the observation of Baer and Fischer (8) that asymmetric triglycerides prepared up to the present time, containing only aliphatic acid residues, show no detectable optical rotation.

IV. PREPARATION OF GLYCEROPHOSPHATES

It has been demonstrated (8) by the synthesis of optically active α-monoglycerides that asymmetrical α-monoglycerides of both plant and animal origin should also show activity when present only in one of the enantiomorphic forms. A causal relationship between optical activity and the asymmetry of the glycerol molecule in naturally occurring glycerides has not yet been established with complete validity. The chief reason for this appears to be the difficulty of isolating pure substances from natural fats, which consist of nearly inseparable mixtures of many glycerides, without altering the original constitution and configuration in the process. Suzuki and Inoue (47) have found that freshly extracted natural fats are optically active and show a declining rotation. Unequivocal proof that this activity results from the asymmetry of the glycerol molecule in natural glycerides has not yet been obtained.

In the case of α-glycerophosphoric acid, which has long been known as a component of the lecithins and cephalins and may in a sense be looked on as a monoglyceride, the situation is much more favorable. Because of its great stability several workers have been able to isolate it from various phosphatides and free it from its accompanying β-isomer (12, 26, 31).

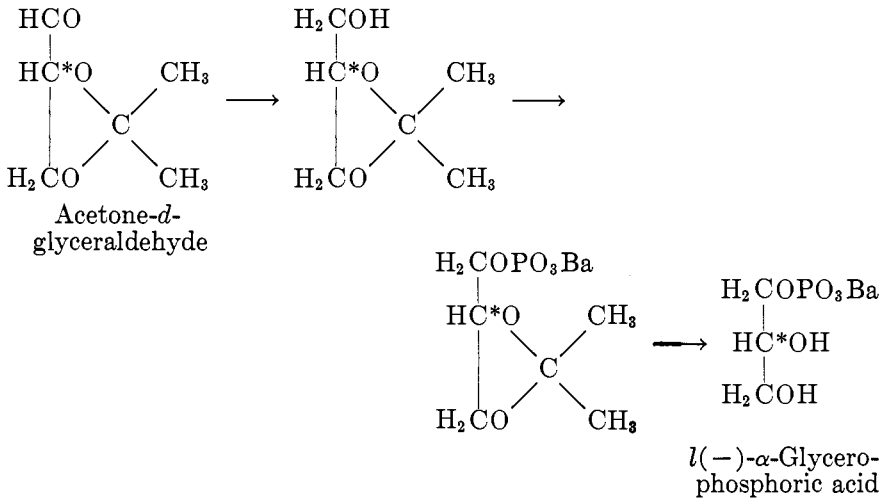
By preparing the comparatively highly active dimethyl ether dimethyl ester of α-glycerophosphoric acid from its barium salt, which shows no perceptible rotation, Karrer and Salomon (31) were able to show conclusively that the α-glycerophosphoric acid in the phosphatides examined

by them is optically active and present in only one enantiomorphic form. Meyerhof and Kiessling (39) later established its identity with the glycerophosphoric acid which forms an intermediate stage in alcoholic fermentation and glycolysis, and observed that the biological *l*(−)-α-glycerophosphoric acid is completely utilized by the enzyme of muscle press juice, while its antipode remains entirely unaltered.

The preparation of the enantiomorphic forms of α-glycerophosphoric acid was undertaken by Abderhalden and Eichwald (3) and by Karrer and Benz (30). However, Abderhalden and Eichwald obtained a non-homogeneous product, while Karrer and Benz were successful only in partially resolving the synthetic α-glycerophosphoric acid of Emil Fischer and Pfähler (23) through the quinine salt, as they showed by comparison of the rotation of the dimethyl ether dimethyl ester of their product with the corresponding derivative of the natural glycerophosphoric acid.

Moreover, the steric relationship between the α-glycerophosphoric acids and compounds of known configuration (active glyceraldehydes, for example) could not be deduced from any information then available. The preparation of the two active α-glycerophosphoric acids was therefore undertaken by the authors, starting from *d*(+)- or *l*(−)-acetoneglycerol, for the purpose of relating their configuration in the sense of Wohl and Freudenberg to that of the active glyceraldehydes.

The sequence of reactions involved was the same as that employed by Emil Fischer and Pfähler (23) for the synthesis of racemic α-glycerophosphoric acid. The enantiomorphic acetoneglycerols were phosphorylated by means of phosphorus oxychloride and quinoline. The protective acetone groups were removed and the α-glycerophosphoric acids isolated as their barium salts. The following equation illustrates the preparation of *l*(−)-α-glycerophosphoric acid from acetone-*d*-glyceraldehyde:



Acetone-*d*-glyceraldehyde

l(−)-α-Glycerophosphoric acid

The steric classification of the two glycerophosphoric acids is established along the same lines of reasoning as discussed above for the aliphatic α-monoglycerides. Thus $d(+)$-acetoneglycerol gives rise to the $l(-)$-α-glycerophosphoric acid, while $l(-)$-acetoneglycerol yields $d(+)$-α-glycerophosphoric acid.

The identity of the synthetic $l(-)$-α-glycerophosphoric acid with the "biological α-glycerophosphoric acid" from glycolysis, alcoholic fermentation, and from phosphatides was established by comparing the rotation of the dimethyl ether dimethyl esters:

(*a*) Synthetic..... $[\alpha]_D = -4.78°$
(*b*) From phosphatides..... $[\alpha]_D = -3.2°$ (31)
(*c*) From glycolysis and fermentation..... $[\alpha]_D^{20°} = -4.46°$ (39)

In agreement with the work of Karrer and Salomon, the barium salt of the synthetic $l(-)$-α-glycerophosphoric acid showed no perceptible rotation. The weak rotations given in the literature for the barium salt of α-glycerophosphoric acid from phosphatides may therefore be ascribed to the presence of optically active impurities. Further, the silver salt of the synthetic acid shows a specific rotation of only $[\alpha]_D = +1.0°$ (in ammoniacal solution). Kiessling and Schuster (32) found $[\alpha]_D = +0.8°$ (saturated aqueous solution) for the silver salt of the natural α-glycerophosphoric acid from fermentation. The diethyl ester of the diethyl ether ($[\alpha]_D^{20°} = -5.31°$, in homogeneous substance) may in future investigations prove to be a valuable preparation for comparison. A rotation of $[\alpha]_D^{20°} = +5.94°$ has been observed for the corresponding derivative of $d(+)$-α-glycerophosphoric acid (11).

V. α-GLYCEROL ETHERS

The occurrence of monoethers of glycerol with higher fatty alcohols, such as octadecyl, cetyl, and oleyl alcohols, in the liver oils of various marine fish, especially those of the Elasmobranch group, was first demonstrated by Tsujimoto and Toyama (50), and later by Nakamiya (40). André and Bloch (5) showed that in the original fish oils these ethers are present in the form of fatty acid esters. From 1928 to 1933 a thorough investigation into the structure of inactive batyl and chimyl alcohols was carried out by Heilbron and Owens (28), Davies, Heilbron, and Owens (17), and Davies, Heilbron, and Jones (18).

Their synthesis (17) of racemic batyl alcohol was accomplished by the condensation of octadecyl chloride with sodium allylate, and subsequent oxidation of the octadecyl allyl ether with perhydrol.

$$CH_2{=}CHCH_2ONa + C_{17}H_{35}CH_2Cl \longrightarrow CH_2{=}CHCH_2OCH_2C_{17}H_{35}$$

Sodium allylate — Octadecyl chloride — Octadecyl allyl ether

$$\xrightarrow[\text{in } CH_3COOH]{H_2O_2} \underset{\text{Racemic batyl alcohol}}{CH_2(OH)-CH(OH)-CH_2OCH_2C_{17}H_{35}}$$

The racemic α-cetyl glycerol ether was prepared in the same way, using cetyl chloride for condensation. These syntheses and the lead tetraacetate titration of the ethers (16) proved that batyl alcohol, chimyl alcohol, and selachyl alcohol must be the α-glycerol ethers of octadecyl alcohol, hexadecyl alcohol, and oleyl alcohol, respectively.

Since substitution of glycerol in the α-position causes the β-carbon atom to become asymmetric, Davies, Heilbron, and Jones (18) were led to examine again the optical properties of natural batyl alcohol. They reported that the natural alcohol has a slight optical activity, $[\alpha]_D^{20^\circ} = +2.6^\circ$ (in chloroform, $c = 0.95$), an observation in disagreement with an earlier finding of Toyama (48).

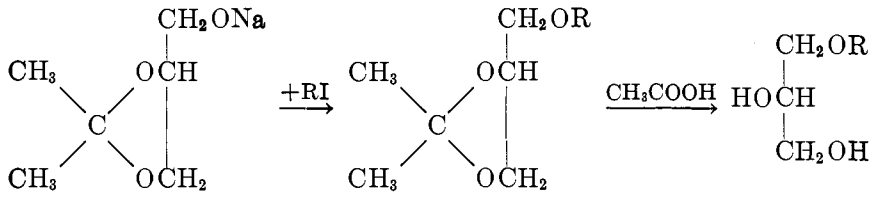
Recently, Toyama and Ishikawa (49) made the observation that the rotations of free batyl and selachyl alcohols in chloroform and ethyl alcohol are largely dependent on the concentrations used. For instance, while the selachyl alcohol in substance showed a specific rotation of $[\alpha]_D^{26^\circ} = -4.33^\circ$, increasing dilution with chloroform or ethyl alcohol resulted in a gradual decrease in value, until at concentrations of approximately 10 per cent no rotation was detectable. When dilutions below 10 per cent were employed a dextrorotation was observed. Similarly, batyl alcohol at a concentration of 3.15 g. per 100 cc. in chloroform solution showed $[\alpha]_D = +2.14^\circ$, while at a concentration of 9.94 g. per 100 cc. in ethyl alcohol the rotation was approximately zero. These observations of Toyama and Ishikawa reconcile the disagreement of Toyama's earlier observation with that of Davies, Heilbron, and Jones (18).

In this laboratory it was found that the two enantiomorphic forms of the synthetic, like the natural, batyl alcohol in a concentration of 10 per cent in chloroform showed no detectable rotation, but their diacetates showed rotations in good agreement with that recorded for the diacetate from natural batyl alcohol (18).

The constitutions of the three natural α-glycerol ethers and the fact that they occurred in one enantiomorphic form were thus well established, although the steric classification of these compounds, or their relationship to glyceraldehyde, was still to be determined. With this object in view, the syntheses of both enantiomorphic forms of the optically active α-glyc-

erol ethers were undertaken, and carried out in such a way that in all stages of the process the optical relations between the resulting ethers and the *d*- and *l*-glyceraldehydes, the reference compounds, could be clearly traced.

The *d*(+)-acetoneglycerol and *l*(−)-acetoneglycerol, which had proved so useful in the synthesis of optically active mono-, di-, and tri-glycerides and glycerophosphates, were again used as starting materials. For the present synthesis, the sodium salts of the acetoneglycerols were brought into reaction with hexadecyl and octadecyl iodides in boiling glycol dimethyl ether (45, 51), yielding the acetone compounds of α-hexadecyl and α-octadecyl glycerols. Hydrolysis with acetic acid gave the free alcohols, which are identical with chimyl alcohol (m.p. 62–63°C.) and batyl alcohol (m.p. 71°C.), respectively, the configuration of which is represented below:



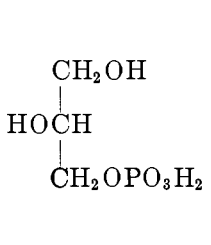
α-Chimyl alcohol: R = $C_{16}H_{33}$
α-Selachyl alcohol: R = $C_{18}H_{33}$
α-Batyl alcohol: R = $C_{18}H_{35}$

The diacetylated synthetic batyl alcohols showed a specific rotation of $[\alpha]_{5461}^{20°} = \pm 8.6°$ (in chloroform, $c = 11.2$). Davies, Heilbron, and Jones had found $[\alpha]_{5461}^{20°} = -8.5°$ (in chloroform, $c = 2.63$) for the natural compound (18). For comparison of the rotation of the acetone compound of natural batyl alcohol with that of the corresponding synthetic compound, a crude preparation of the glycerol ethers from the unsaponifiable fraction of ratfish (*Chimaera Monstrosa*) liver oil (m.p. 61.5–62.5°C., containing mainly chimyl alcohol) was acetonated. An acetone compound was obtained with the specific rotation of $[\alpha]_D = -14.0°$ (in substance). The two acetone compounds of the synthetic chimyl and batyl alcohols had specific rotations of $[\alpha]_D = \pm 12.1°$ and $[\alpha]_D^{40°} = \pm 12.6°$, respectively (in melted substance). Since the rotations of derivatives of the natural batyl alcohol agree with those of the derivatives of α-octadecylglycerol synthesized from *l*(−)-acetoneglycerol, it must be concluded that the batyl alcohol belongs to the *d*-series (see above). The steric relationship was assigned according to the principles used for the classification of the α-monoglycerides and glycerophosphates (page 299 of this article). Selachyl alcohol also belongs to the *d*-series, because it can be transformed by catalytic reduction to *d*-batyl alcohol (50).

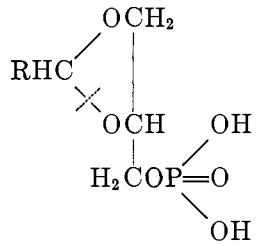
Polarimetric examination of synthetic chimyl alcohol in chloroform at

a concentration of 10 per cent showed no perceptible rotation, in agreement with the finding of Toyama and Ishikawa (49). However, the acetone compound and the diacetyl derivative of the synthetic product prepared from *l*(−)-acetoneglycerol showed a rotation of the same sign as that of natural chimyl alcohol. Therefore natural chimyl alcohol belongs to the *d*-series, for the same reason that batyl alcohol does.

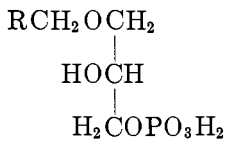
It is conceivable that these ethers (IV) are formed in the cell by reductive splitting of the acetal phosphatides (plasmals) (19), II → III,



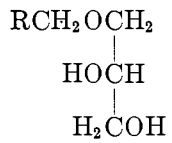
I
Glycerophosphoric acid



II



III



IV

and subsequent removal of phosphoric acid, III → IV. The fact that the natural glycerophosphoric acid (I) belongs to the *l*-series, and that the glycerol ethers, as far as they have been investigated, belong to the *d*-series, is in agreement with the above hypothesis.

In other words, it appears that etherification and esterification take place in nature in the α- and α'-positions, respectively, which in asymmetrically substituted glycerols are no longer equivalent.

It is also interesting to point out that the glycerol acetals with fatty acid aldehydes might be related to the corresponding fatty acid glycerides by an oxidation and reduction mechanism. Thus it would not be surprising if future investigation reveals that these glycerol acetals play an important rôle in fat metabolism.

VI. BIOCHEMICAL CHARACTERISTICS OF THE ENANTIOMORPHS

In most cases it would be difficult to follow, in intact animals, the metabolism of enantiomorphic compounds of the types considered here, but it was felt that some indication of the behavior of these enantiomorphs in the animal body might be obtained by studying the action of enzymes

on these compounds in isolated systems. Investigations to establish the significance of optical asymmetry were therefore begun, using various glycerol derivatives, such as glycerophosphates, mono-, di-, and triglycerides and glycerol ethers and acetals with fatty acid residues.

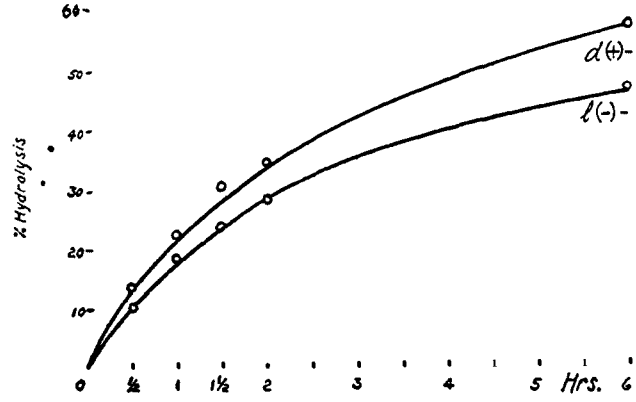


FIG. 1. Hydrolysis of $l(-)$- and $d(+)$-α-glycerophosphates by pig kidney phosphatase. 8 cc. of ammonium buffer (pH, 9.6), 8 cc. of substrate (containing 8.0 mg. of organic phosphorus), and 4 cc. of enzyme solution were used.

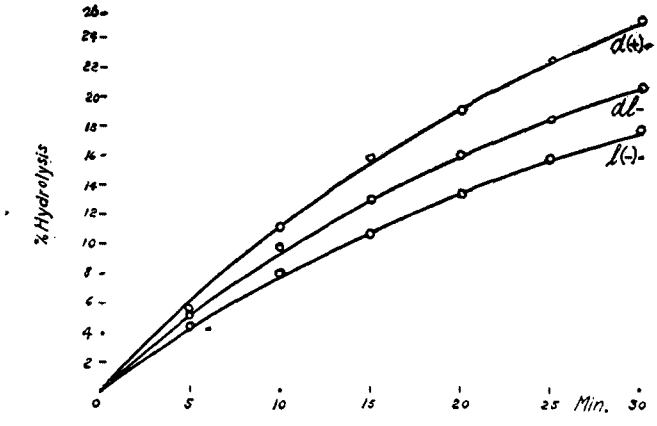


FIG. 2. Hydrolysis of inactive, $l(-)$-, and $d(+)$-α-glycerophosphates by phosphatase prepared from dog feces. 25 cc. of carbonate–veronal buffer (pH, 8.62), 20 cc. of substrate (containing 6.2 mg. of organic phosphorus), and 5 cc. of enzyme solution were used.

A. Phosphatases

A study of the action of phosphatases on glycerophosphates was undertaken first, because the glycerophosphates are less liable to acyl migration

than glycerides with fatty acid residues, and because it was already known that the two enantiomorphic α-glycerophosphates react differently with the ferments of muscle press juice. Meyerhof and Kiessling (39) found, using the racemic compound as substrate, that only the *l*(−)-α-glycerophosphoric acid was used up completely, the *d*(+)-α-glycerophosphoric acid remaining unchanged.

Experiments repeated by Meyerhof[6] with samples of the synthetic pure *l*(−)- and *d*(+)-α-glycerophosphoric acids have confirmed the previous result.

The rate of hydrolysis of *l*(−)-α-glycerophosphoric acid and *d*(+)-α-glycerophosphoric acid by unrefined kidney phosphatase, taka-phosphatase, rat bone phosphatase, and a purified phosphatase from dog feces has now been investigated (11). It was shown with all four preparations that, under the conditions specified, the *d*(+)-α-glycerophosphoric acid was hydrolyzed with greater velocity than the *l*(−)-α-glycerophosphoric acid. This fact has probably some physiological significance.

Typical results obtained with two enzymes—namely, kidney phosphatase with ammonium buffer (pH, 9.6) and phosphatase from dog feces with carbonate–veronal buffer (pH 8.62)—are illustrated by the curves shown in figures 1 and 2. The method used to follow the rate of hydrolysis was the same as that described by King and Armstrong (35), and the determination of free phosphoric acid was carried out according to King (34).

The racemic compound is hydrolyzed, as was to be expected, at a rate approximately midway between those of its two components. Taka-phosphatase with phthalate buffer (pH, 3.8) and rat bone enzyme with carbonate–veronal buffer (pH, 8.62) gave essentially similar results.

B. Lipases[7]

In contrast to the α-glycerophosphates, which show no tendency to migrate, the glycerides containing fatty acid residues are known to be quite susceptible to migration. For this reason two kinds of derivatives of α-monoglycerides, in which migration of the acyl residue in alkaline media was made impossible by substituting the β- and α′-hydroxyl groups, were chosen to study the action of lipases.

The investigation was first carried out with the acetone compounds of stereoisomeric α-butyryl- and α-caproylglycerols. The results are given in figure 3.

It is apparent from the curves that, under the experimental conditions

[6] Private communication.

[7] Unpublished results of Erich Baer and H. O. L. Fischer.

used, the acetone compounds of the *d*-series (prepared from *l*(−)-acetoneglycerol) were hydrolyzed by the lipase of the guinea pig serum with approximately twice the velocity observed for the corresponding antipodes. The velocity of hydrolysis of the racemic compounds is just intermediate between the velocities observed for the pure isomers, thus substantiating these latter values.

Analogous experiments were carried out with the dimethyl ethers of the enantiomorphic α-butyrylglycerols as substrates. The blood serum and liver extract of guinea pigs, rabbits, and rats were used as a source of lipase. Under the conditions used (0.04 molar substrate solution; pH,

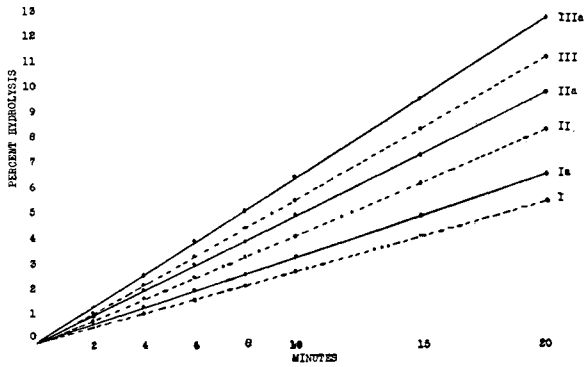


FIG. 3. Hydrolysis of the acetone compounds of the enantiomorphic α-butyryl- and α-caproyl-glycerols. Curve I, acetone compound of *l*(−)-α-*n*-butyrylglycerol; curve II, acetone compound of inactive α-*n*-butyrylglycerol; curve III, acetone compound of *d*(+)-α-*n*-butyrylglycerol; curve Ia, acetone compound of *l*(−)-α-*n*-caproylglycerol; curve IIa, acetone compound of inactive α-*n*-caproylglycerol; curve IIIa, acetone compound of *d*(+)-α-*n*-caproylglycerol. The hydrolysis was carried out by the "continuous titration" technique at pH 7.2 in a mixture containing 10 cc. of 0.1 *M* substrate solution (in gum acacia), 10 cc. of water, 1 cc. of phenol red indicator, and 0.4 cc. of guinea pig serum.

7.2; temperature, 37°C.; "continuous titration"), it was found that one enantiomorph, in this case the dimethyl ether of the *l*(−)-α-butyrylglycerol, was hydrolyzed with a velocity 1.2 to 3.5 times higher than the other.

These two series of experiments, using acetonated and methylated monoglycerides as substrates, clearly demonstrate that the asymmetry of the β-carbon atom has an influence on the action of enzymes on glycerides.

The free enantiomorphic monoglycerides are, in many instances, difficult to prepare in an optically pure state, and are easily racemized. However,

investigation has shown that the free enantiomorphic α-butyrins are hydrolyzed with different velocities by the lipase of guinea pig serum. The observed difference is largely dependent on concentrations of the substrates, as is shown by the following example: In 0.3 molar substrate solution one α-monobutyrin was hydrolyzed with a velocity 14 per cent greater than its antipode, while in 0.05 molar substrate solution a difference in velocity of hydrolysis of 85 per cent was observed.

Further investigations regarding enzyme action on synthetic enantiomorphic glycerol derivatives are being carried out in this laboratory.

VII. BIOLOGICAL CONCLUSIONS TO BE DRAWN FROM THE CONFIGURATION OF THE NATURAL α-GLYCEROPHOSPHORIC ACID

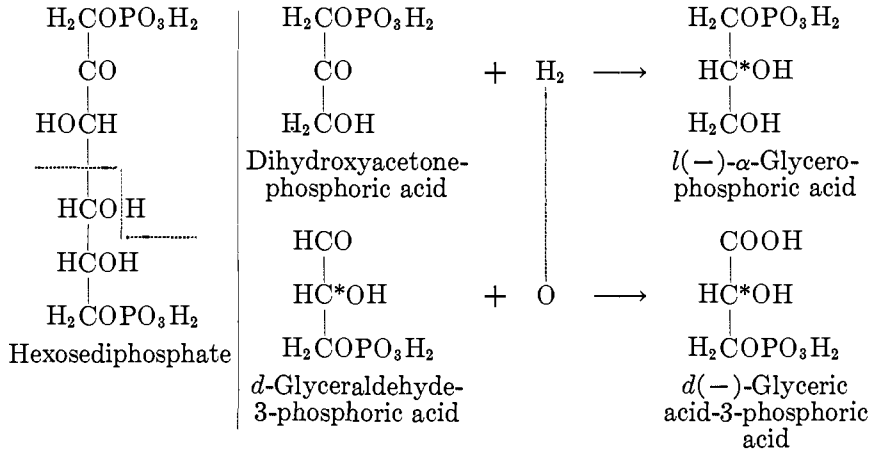
Once the steric relationship of the α-glycerophosphoric acids to the glyceraldehydes has been fixed by synthesis from the two enantiomorphic acetoneglycerols, certain biological conclusions may be drawn.

In the fermentation scheme of Embden, as developed by Meyerhof, Kiessling, and Lohmann, the dismutation of triosephosphoric acids plays an important part. Since α-glycerophosphoric acid is the product of simple reduction of triosephosphoric acids, it follows from our purely chemical evidence that the natural *l*(−)-α-glycerophosphoric acid does not arise biologically from the natural *d*-glyceraldehyde-3-phosphoric acid, because the latter belongs to another steric series, but that it must have been formed by asymmetrical fermentative reduction from dihydroxyacetonephosphoric acid.

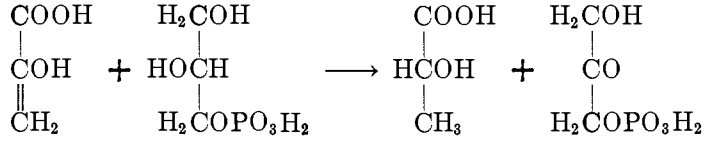
The steric configuration of *l*(−)-α-glycerophosphoric acid having been established by synthesis (10), it became a comparatively simple matter to determine the steric configuration and consequently the steric relationship between the biological α-glycerophosphoric acid from yeast and the natural levorotatory glyceric acid-3-phosphoric acid.

Kiessling and Schuster (32) oxidized the biological *l*(−)-α-glycerophosphoric acid with bromine and obtained, as might be predicted from the relationship already established by synthesis, not the natural glyceric acid-3-phosphoric acid but its antipode *l*(+)-glyceric acid-3-phosphoric acid, which has no counterpart in nature.

This is further evidence that the two dismutation products of triosephosphoric acids, α-glycerophosphoric acid and glyceric acid-3-phosphoric acid, belong to two different steric series. Thus the natural glyceric acid-3-phosphoric acid must belong to the *d*-series and accordingly can arise by oxidation only from *d*-glyceraldehyde-3-phosphoric acid. Summing up these results, we obtain the accompanying scheme for the dismutation of triosephosphoric acids:



In the light of this new steric relationship the well-known Embden–Meyerhof reaction of pyruvic acid and α-glycerophosphoric acid, which has hitherto been assumed to yield lactic acid and *d*-glyceraldehyde-3-phosphoric acid, must now be written thus:



The fact that *l*-glyceraldehydephosphoric acid is not utilized biologically (as has been demonstrated from experience with *d*,*l*-glyceraldehyde-3-phosphoric acid) compels the conclusion that dihydroxyacetonephosphoric acid must be formed in this reaction.

Both enantiomorphic α-glycerophosphoric acids are ideal starting materials for the synthesis of phosphatide acids and phosphatides of which the configuration is known with regard to the β-carbon atom of the glycerol residue. Since only the *l*(−)-α-glycerophosphoric acid has so far been found in natural substances, it appears likely that most of the α-phosphatides which have as yet been investigated are derived from this acid, rather than from its antipode, and consequently the configuration of the glycerol part of these phosphatides is established.

It is probable that an important rôle is played by *l*(−)-α-glycerophosphoric acid in the transformation of carbohydrate into fat in the bodies of higher animals. It seems to be the substance which, formed from phosphorylated sugars, in fermentation and glycolysis, would supply the asymmetry for the synthesis of optically active α-phosphatides and fats in nature.

REFERENCES

(1) ABDERHALDEN, E., AND EICHWALD, E.: Ber. **47**, 1856 (1914).
(2) ABDERHALDEN, E., AND EICHWALD, E.: Ber. **48**, 113, 1851 (1915).
(3) ABDERHALDEN, E., AND EICHWALD, E.: Ber. **51**, 1308 (1918).
(4) ANDERSON, E., AND SANDS, L.: *Organic Syntheses*, Collective Volume I, p. 60 John Wiley and Sons, Inc., New York (1932).
(5) ANDRÉ, É., AND BLOCH, A.: Compt. rend. **195**, 627 (1932).
(6) BAER, E., AND FISCHER, H. O. L.: J. Am. Chem. Soc. **61**, 761 (1939).
(7) BAER, E., AND FISCHER, H. O. L.: J. Biol. Chem. **128**, 463 (1939).
(8) BAER, E., AND FISCHER, H. O. L.: J. Biol. Chem. **128**, 475 (1939).
(9) BAER, E., AND FISCHER, H. O. L.: J. Biol. Chem. **128**, 480 (1939).
(10) BAER, E., AND FISCHER, H. O. L.: J. Biol. Chem. **128**, 491 (1939).
(11) BAER, E., AND FISCHER, H. O. L.: J. Biol. Chem. **135**, 321 (1940).
(12) BAILLY, O.: Compt. rend. **160**, 395, 663 (1915); Rev. gén. sci. **29**, 208 (1918); Bull. soc. chim. biol. **1**, 152 (1919).
(13) BERGMANN, M., AND SABETAY, S.: Z. physiol. Chem. **137**, 47 (1924).
(14) BERGMANN, M., BRAND, E., AND DREYER, F.: Ber. **54**, 936 (1921).
(15) BRIGL, P., AND GRUNER, H.: Ber. **67**, 1969 (1934).
(16) CRIEGEE, R.: Ann. **481**, 263 (1930); Ber. **64**, 260 (1931).
(17) DAVIES, G. G., HEILBRON, I. M., AND OWENS, W. M.: J. Chem. Soc. **1930**, 2542.
(18) DAVIES, W. H., HEILBRON, I. M., AND JONES, W. E.: J. Chem. Soc. **1933**, 165.
(19) FEULGEN, R.: Z. physiol. Chem. **180**, 170 (1929).
FEULGEN, R., AND BEHRENS, M. B.: Z. physiol. Chem. **256**, 15 (1938).
FEULGEN, R., AND GRÜNBERG, H.: Z. physiol. Chem. **257**, 161 (1939).
(20) FISCHER, EMIL: Ber. **23**, 370 (1890).
(21) FISCHER, EMIL: Ber. **53**, 1621 (1920).
(22) FISCHER, E., AND BRAUNS, F.: Ber. **47**, 3181 (1914).
(23) FISCHER, E., AND PFÄHLER, E.: Ber. **53**, 1606 (1920).
(24) FISCHER, E., BERGMANN, M., AND BÄRWIND, H.: Ber. **53**, 1589 (1920).
(25) FISCHER, H. O. L., AND BAER, E.: Naturwissenschaften **25**, 588 (1937).
(26) GRIMBERT, L., AND BAILLY, O.: Compt. rend. **160**, 207 (1915).
(27) GRÜN, A., AND LIMPÄCHER, R.: Ber. **60**, 255 (1927).
(28) HEILBRON, I. M., AND OWENS, W. M.: J. Chem. Soc. **1928**, 942.
(29) JACKSON, D. T., AND KING, C. G.: J. Am. Chem. Soc. **55**, 678 (1933).
(30) KARRER, P., AND BENZ, P.: Helv. Chim. Acta **9**, 23 (1926).
(31) KARRER, P., AND SALOMON, H.: Helv. Chim. Acta **9**, 3 (1926).
(32) KIESSLING, W., AND SCHUSTER, P.: Ber. **71**, 123 (1938).
(33) KILIANI, H.: Ber. **55**, 100 (1922); **58**, 2349 (1925).
(34) KING, E. J.: Biochem. J. **26**, 292 (1932).
(35) KING, E. J., AND ARMSTRONG, A. R.: Can. Med. Assoc. J. **31**, 376 (1934); **32**, 379 (1935).
(36) KNORR, L., AND KNORR, E.: Ber. **32**, 753 (1899).
(37) LEVENE, P. A., AND WALTI, A.: J. Biol. Chem. **73**, 263 (1927).
(38) MALAPRADE, L.: Bull. soc. chim. **43**, 683 (1928).
(39) MEYERHOF, O., AND KIESSLING, W.: Biochem. Z. **264**, 46, 62 (1933); **267**, 330 (1933).
(40) NAKAMIYA, Z.: Bull. Inst. Phys. Chem. Research (Tokyo) **17**, 837–852 (1938); Chem. Abstracts **33**, 8175 (1939).
(41) ROSENHEIM, A., AND TEBB, A. C.: J. Physiol. **37**, 348 (1908).
(42) RUPE, H., AKERMANN, A., AND TAKAGI, H.: Helv. Chim. Acta **1**, 452 (1918).
(43) SANO, M.: J. Biochem. **1**, 17 (1922).

(44) SCHOENHEIMER, R.: Harvey Lectures, 1936–37.
(45) SCOTT, N. D., WALKER, J. F., AND HANSLEY, V. L.: J. Am. Chem. Soc. **58**, 2442 (1936).
(46) SUZUKI, B.: Proc. Imp. Acad. Japan **7**, 230 (1931); Chem. Zentr. **1931, II**, 2345.
(47) SUZUKI, B., AND INOUE, Y.: Proc. Imp. Acad. (Tokyo) **6**, 71 (1930); Chem. Abstracts **24**, 4265 (1930); Chem. Zentr. **1930, II**, 1063.
(48) TOYAMA, Y.: Chem. Umschau **31**, 61 (1924).
(49) TOYAMA, Y., AND ISHIKAWA, T.: J. Chem. Soc. Japan **59**, 1367 (1938).
(50) TSUJIMOTO, M., AND TOYAMA, Y.: Chem. Umschau **29**, 27, 237 (1922); **31**, 13, 61, 135, 153 (1924).
(51) WALKER, J. F., AND SCOTT, N. D.: J. Am. Chem. Soc. **60**, 951 (1938).
(52) WOHL, A., AND FREUDENBERG, K.: Ber. **56**, 309 (1923).