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REVIEW

Preparation of Labelled Lipids

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SUMMARY

In the present review emphasis is placed on the description of advances made in the synthesis and biosynthesis of labelled neutral lipids as well as phosphatides and other complex ionic lipids. Methods applicable to the analysis and purification of these compounds are described briefly. In addition observations related to the autoradiolysis of labelled lipids during storage are discussed.

Much progress in the chemistry of lipids has been made as a result of the development of efficient separation techniques. Applications of chromatographic methods to the analysis of commercially available labelled lipids have revealed shortcomings of many procedures for the preparation of these compounds. Accordingly, improved procedures and new routes of synthesis have been devised. Chromatography has facilitated the isolation of uniformly labelled fatty acids as well as phospholipids, sulfolipids and glycolipids from microorganisms grown in radioactive media.

SYNTHESIS.

Fatty Acids : Advances made in the synthesis of labelled fatty acids have been reviewed recently [1, 2]. Labelled saturated and unsaturated fatty acids of different chain lengths are being offered commercially [3]; thus facilitating the preparation of various lipids tagged in the long aliphatic chains. However, commercial fatty acids should be thoroughly analyzed and, if necessary, purified before being used as starting materials in syntheses of other lipid compounds.

Ester Lipids : 1-Monoglycerides, 1,3-diglycerides and triglycerides can be synthesized by a method in which the glycerol moiety is built up stepwise.

Thus, the alcoholic groups of glycerol become available successively to react with fatty acids [4]. This route of synthesis is applicable to the preparation of glycerides labelled in the glycerol moiety, in either one, two or three of the acyl groups, or in both the glycerol and the fatty acid moieties [4]. Triglycerides containing identical acyl groups can be readily prepared by transesterifying an unlabelled triglyceride with a labelled fatty acid [5] or by reacting glycerol with a fatty acid using sulfuric acid as a catalyst [6]. Esterification is accomplished more efficiently by using the mixed anhydride of a fatty acid with trifluoroacetic acid as acylating agent [7]. The esterification of glycerol, or another polyhydric alcohol with an acid chloride can be carried out in pyridine-chloroform [8, 9]; a labelled acid chloride can be conveniently prepared by equilibrating a labelled acid with the corresponding "cold" acid chloride in chloroform solution [10].

Wax esters and cholesteryl esters are obtained by reacting a long-chain alcohol or cholesterol, respectively, with an acid chloride in an anhydrous solvent [11]. The same compounds can be prepared in good yields by the transesterification of either alkyl acetates or cholesteryl acetate with methyl esters in the presence of sodium ethoxide [12].

Unusual Neutral Lipids : Research on the biological functions and the metabolism of minor classes of lipids depends largely on the use of labelled compounds. Alkyl methanesulfonates ("mesylates"), which can be obtained conveniently in good purity and high yield [13, 14], are eminently suitable for the preparation of labelled saturated and unsaturated hydrocarbons, aldehydes, ethers and intermediates for the synthesis of other unusual lipids. Mesylates of saturated and unsaturated alcohols are also of great value for the preparation of labelled fatty acids by chain elongation.

Experimental conditions employed in the preparation of various labelled lipids from mesylates are summarized in Table 1. In the same table, reference is made to some alternative routes of synthesis and to further reactions and products.

Most of the older methods for the synthesis of unusual lipids are not applicable to the preparation of unsaturated compounds either because they involve hydrogenation procedures or because they lead to isomerization of double bonds.

Unsaturated compounds can be used for the preparation of ^{3}H-labelled saturated [27, 28] and unsaturated [29, 30] substances by total or stereospecific partial hydrogenation with tritium gas. Morevover, unsaturated lipids can serve as intermediates in the synthesis of naturally occurring compounds containing cyclopropane rings [31] and for the preparation of substances labelled with ^{125}I or ^{131}I [32].

Phospholipids, Sulfolipids and Glycolipids : The synthesis of a ^{3}H-labelled lysophosphatidic acid has been described [33]. A few lecithins and other phospholipids labelled with ^{3}H [34], ^{14}C [34, 35] or ^{32}P [34] have also been prepared. In a recent publication a procedure has been described for the preparation

TABLE 1. Reactions of Aliphatic Methanesulfonates, $R\text{-}CH_2\text{-}O\text{-}SO_2\text{-}CH_3$

Experimental Conditions	Products	Yield %	(Ref.)	Alternative Routes (Ref.)	Further Reactions and Products	(Ref.)
$LiAlH_4$ → Diethyl ether, 35° C, 6 hr	*Hydrocarbons*	> 95	[14,15]	[16]		
$MgBr_2$/Diethyl ether → Diethyl ether, 20° C, 24 hr	*Bromides*	> 95	[14,17]	[18]	Grignard Reactions → Hydrocarbons prim; sec. tert. Alcohols Aldehydes Ketones Acids	[8]
KCN → Dimethyl sulfoxide, 80° C, 1 hr	*Nitriles*	> 95	[19]	[20]	Saponification → Fatty acids	[19]
Dimethyl sulfoxide, $NaHCO_3$ → 170° C, 10 min	*Aldehydes*	60-70	[21]	[22,23]	→ Alk-1-enyl ethers	
Isopropylidene glycerol-K → Xylene, 140° C, 4 hr	*Alkyl glycerol ethers*	80-90	[13,14]	[7]	Further alkylation → Dialkyl and Trialkyl glycerol ethers	[24,25]
Diethyl malonate-Na → Ethanol, 80° C, 2 hr	*Fatty Acids*	> 90	[14]	[26]		

of ^{35}S-labelled cerebroside sulfates from cerebrosides that had been isolated from beef brain [36]. Syntheses of several ^{14}C-labelled glycolipids [37, 38] and the preparation of a tritiated ceramide trihexoside [39] have been reported.

The chemical synthesis of specifically labelled phospholipids and other complex lipids will certainly receive considerable attention in the future.

BIOSYNTHESIS.

Fatty Acids : Labelled fatty acids have been isolated from cultures of the algae *Chlorella pyrenoidosa* [40] and *Euglena gracilis* [41], the fungus *Phycomyces blakesleeanus* [42] and the protozoon *Ochromonas danica* [43]. Soybean plants [44] which were cultivated in the presence of $^{14}CO_2$ or $^{3}H_2O$, and perilla plants [45] grown in an atmosphere of $^{14}CO_2$ have been used for the biosynthesis of uniformly labelled fatty acids. Radioactively tagged fatty acids have been isolated also from the seeds that had developed in cotton bolls into which ^{14}C-labelled glucose had been introduced during maturation [46].

Ester Lipids : Regrettably, in all of the above mentioned studies, the total lipids obtained from the microorganisms or higher plants were hydrolyzed because the only aim was to isolate labelled fatty acids.

Methods have become available for the resolution of complex mixtures of neutral lipids into classes of compounds, and for the further fractionation of each of these classes into individual compounds. With the aid of chromatographic techniques it should now be possible to isolate individual triglycerides and other neutral lipids from microorganisms and plants cultivated in an atmosphere of $^{14}CO_2$ and from small animals reared on diets containing labelled compounds.

Unusual Neutral Lipids : The potential of biosynthetic methods for the production of tagged unusual neutral lipids has hardly been exploited. Only a few isoprenoid compounds, such as uniformly labelled phytol [47] and β-carotene [48], have been isolated from the nonsaponifiable lipid fraction of microorganisms that were grown in radioactive media. Labelled squalene [49] has been obtained from pig livers after perfusion with ^{14}C-labelled acetate.

Phospholipids, Sulfolipids and Glycolipids : Germinating soybeans [50] and rat liver slices [34] have been used for the preparation of labelled phospholipids. The preparation of sphingolipids by biosynthesis appears feasible [51].

Only recently uniformly labelled phospholipids have become available commercially. They were isolated from cultures of the alga *Chlorella pyrenoidosa* that were propagated on an inorganic medium; $^{14}CO_2$ being the sole source of radioactive carbon.

These preparations are pure only with respect to class because methods for fractionating molecular species of phospholipids, sulfolipids and glycolipids are not available.

Chlorella pyrenoidosa is of great value in the preparation of uniformly labelled lipids. This alga is rather insensitive to ^{14}C-radiation and can therefore

be used for the preparation of compounds having high specific activities. Under certain conditions, *Chlorella pyrenoidosa* produces lipids up to 70 % of its dry weight. Besides triglycerides and phospholipids, the lipid fraction of this organism contains significant amounts of sulfolipids and large proportions of various glycolipids [52]. The constituent fatty acids of these lipid classes are of chain lengths C_{16} and C_{18}, saturated, mono-unsaturated, di-unsaturated and tri-unsaturated acids being present in both series.

Possible sources of labelled lipids that have not been exploited are *cell cultures*, *tissue cultures* and *perfused organs*. The formation of certain lipids from labelled precursors can be promoted by modifications of the culture conditions of microorganisms and plants, by changes in the dietary regime of animals, and by the use of drugs [53].

Analysis and Purification of Labelled Lipids.

Chromatography on thin layers of silicic acid is the method of choice for the fractionation of lipids into classes of compounds on an analytical as well as on a preparative scale [47, 54]. As adsorption chromatography does not, as a rule, resolve mixtures of saturated and unsaturated compounds having the same type and number of functional groups, it is essential that techniques based upon complementary principles be employed consecutively. Methods that complement adsorption chromatography include argentation chromatography on layers of silicic acid impregnated with silver nitrate [54, 55], reversed-phase partition chromatography on layers of Kieselguhr [14] or on paper [47] both impregnated with paraffin or silicone, and gas-liquid chromatography [56, 57]. Countercurrent distribution [40, 44] and zone refining [58] also are useful for purifying labelled lipids.

Stability of Labelled Lipids During Storage.

Radiation-induced self-decomposition is pronounced with labelled steroids. It has been claimed that impurities found in preparations of labelled aliphatic lipids are also produced by radiolysis. However, analyses of commercial labelled lipids have revealed [47, 59] that most of the impurities are intermediates and side-products in the synthesis of these compounds and, occasionally, autoxidation products. In contrast to ^{14}C-labelled steroids, aliphatic lipids are remarkably stable. Thus, preparations of oleic, linoleic and linolenic acids, uniformly labelled with ^{14}C, did not show any decomposition after having been stored under nitrogen, in sealed ampoules, at —40° C for ten years [60].

As preparations of unsaturated lipids are subject to autoxidation, they should be kept in solution, under nitrogen, sealed in ampoules and stored in a freezer.

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