Chemical Synthesis of Sphingolipids

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Abstract

The chemical synthesis of sphingosine, derivatives of sphingosine, and sphingolipids are reviewed.

THE PAST TWO DECADES have seen rapid progress in the study of sphingolipids. The exact structure of sphingosine, including its stereochemistry has been elucidated. The structures of sphingomyelin and the cerebrosides have been firmly established and new, more effective methods of isolation and purification of lipids have been developed. These achievements have paved the way for the synthetic studies described in this paper.

The sphingolipids of established structure are: the parent base sphingosine, *trans*-D-*erythro*-1, 3-dihydroxy-2-amino-4-octadecene; the sphingomyelins (IV) in which the primary hydroxyl is attached to phosphorylcholine, while the amino group forms an amide with a long chain fatty acid; the cerebrosides (VIII, IX) in which the primary hydroxyl is glycosidically linked to galactose and which can be hydrolyzed to psychosine (XI). A disaccharide of N-acylsphingosine known as cytolipin H (XII) has recently been isolated from human epidermoid carcinoma (1) and has aroused considerable interest because of its immunological properties. Cytolipin H may be considered as a fragment of the ganglioside molecule (XIII). The structures of some gangliosides have recently been proved with reasonable certainty by Klenk (2) and later by Kuhn (3). The remarkable biological and chemotherapeutical properties which the gangliosides exhibit (4) promise to make them one of the most interesting areas in lipid research.

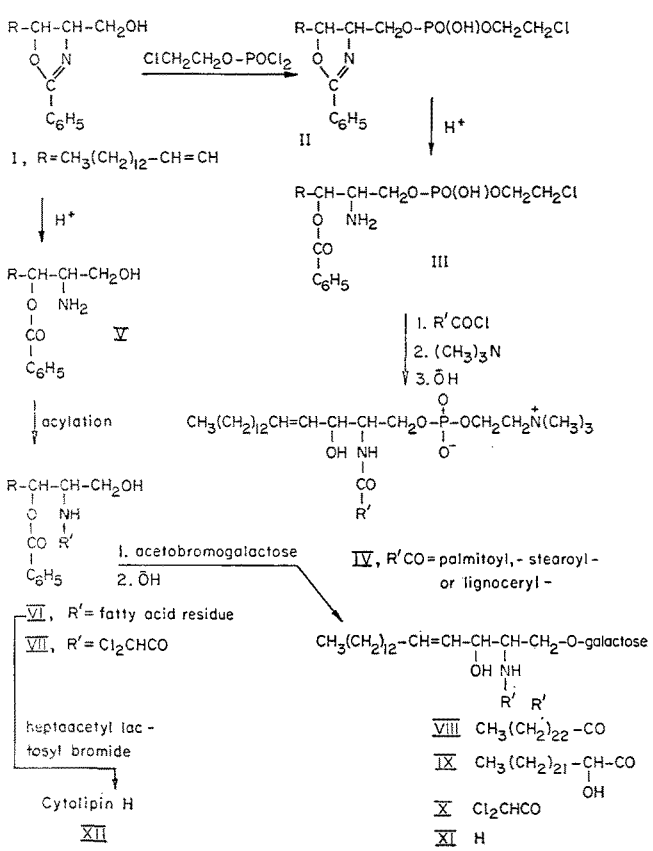

FIG. 1

It is the purpose of my lecture to discuss the synthetic work on the sphingolipids done in our laboratories for the past several years.

The accomplishment of the synthesis of sphingosine in 1954 (5) rendered possible a synthetic approach to its more complex derivatives. Of course, the presence in the molecule of sphingosine of three functional groups and their proper selective protection posed a problem. This was overcome by employing as starting material the substituted oxazoline (I) which could be obtained from an intermediate in the synthesis of sphingosine (6). Thus, the synthesis of sphingomyelin proceeds via the following steps; phosphorylation of I is carried out with β-chloroethylphosphoryl dichloride. The phosphate ester (II) formed is treated with acid whereby the oxazoline ring is opened to give the amine (III). The appropriate fatty acid is then introduced and the resulting amide is treated with trimethylamine to effect quaternization. Hydrolysis of the protecting benzoyl group and replacement of the chlorine atom by OH by treatment with an anion exchange resin leads to racemic sphingomyelin (6).

The finding of Sribney and Kennedy (7) that the enzymatic synthesis of sphingomyelin requires the *threo* form of the sphingosine moiety made desirable a revision of the accepted erythro configuration which had never been proved. To this end a total synthesis of sphingomyelin was developed (8) which employed the D-isomer of the disubstituted sphingosine (VI). This key intermediate was obtained by acylation of V whose optical resolution was effected by means of tartaric acid. Phosphorylation of VI led to D-sphingomyelin which proved identical with the natural lipid. This result reaffirmed the erythro configuration for natural sphingomyelin.

The disubstituted sphingosine VI has also served as a convenient starting material for the synthesis of cerebrosides. Its Koenigs-Knorr reaction with aceto-

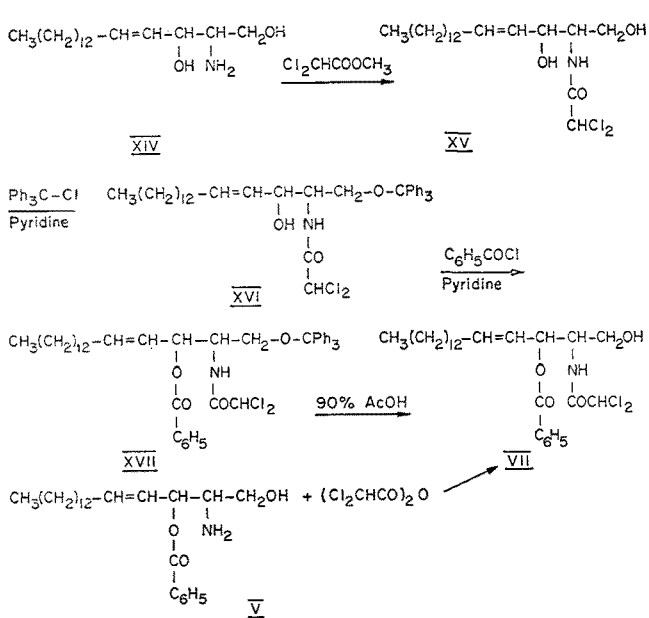

FIG. 2

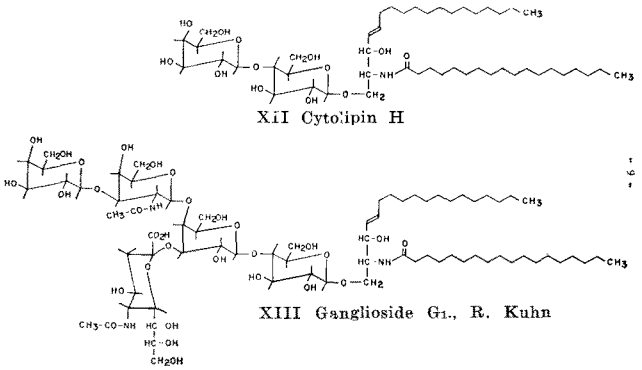

FIG. 3

bromogalactose led to the galactosides cerasine (VIII) and phrenosine (IX) and to the Gaucher's glucocerebroside (9). In a similar fashion, using heptaacetyl lactosyl bromide, cytolipin H was synthesized (10). The synthetic product proved identical with the natural one in its immunological properties.

We shall now discuss a study which led to the synthesis of psychosine (XI). The hydrolytic cleavage of the fatty acids from cerebrosides is known to proceed sluggishly. Therefore, in the synthesis of psychosine the protecting N-acyl group must be such as to undergo mild hydrolysis. In a search for suitable protection of the amine function we prepared a series of galactosides in order to study the conditions by which they can be converted in psychosine. These galactosides differed from the normal cerebrosides by having an activated amide forming group instead of the fatty acid residue. Of the protective groups investigated the dichloroacetyl group provided a smooth route to psychosine (11). The dichloroacetyl cerebroside (X) could be obtained in high yield by glycosidation of VII. The latter compound was synthesized as outlined in Figure 2.

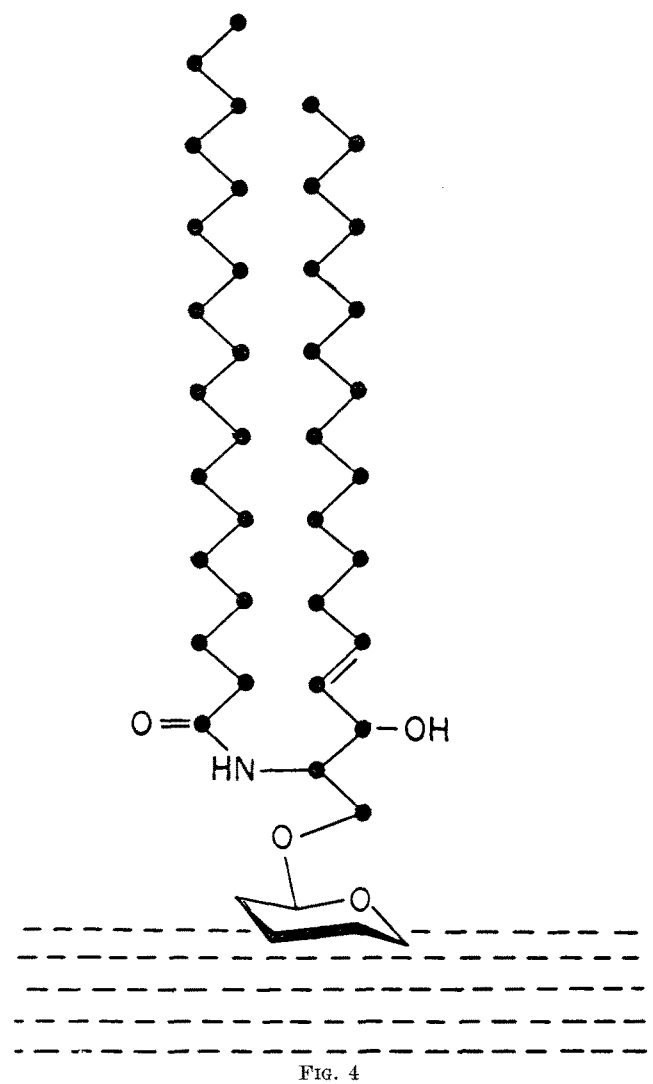

FIG. 4

The hydrolysis of the dichloroacetyl cerebroside (X) to psychosine did not appear at first to be feasible. When a dioxane solution (and I would like to stress the word *solution*) was heated with barium hydroxide following the accepted method introduced by Carter and Fujino (12), the hydrolysis was accompanied by extensive cleavage of the glycosidic bond, leading to a negligible yield of impure psychosine. This behaviour must be attributed to the strong negative nature of the dichloroacetyl grouping which renders this cerebroside alkali sensitive. It is well established that alkali sensitivity of glycosides is a function of the aglycon. It may withdraw electrons from the glycosidic bond as a result of activation by substituents in the β-position. The electron attraction inherent in the dichloroacetamido group might exercise such an effect. In looking for a way by which we could interfere with the ionic mechanism of cleavage, the following train of thought was helpful. The molecule of a cerebroside consists of a hydrophobic and a hydrophilic part (Fig. 4). In analogy to the behaviour of higher fatty acids, a suspension of the cerebroside on water might form a solid and a liquid film consisting of a unimolecular layer with the lipid portion floating on the surface and the sugar moiety in the surface of water. Such a situation, we reasoned, would impede ionic intereaction between the two parts of the molecule and perhaps facilitate hydrolysis without affecting the glycosidic linkage. Accordingly, we returned to the method of Klenk using aqueous barium hydroxide (13). In fact, when a *suspension* of the dichloroacetylcerebroside (X) was warmed with 5% aqueous barium hydroxide a quick change of texture of the solid could be observed, and after one hour psychosine was obtained in 80% yield. This method also enabled us to synthesize lactosylsphingosine.

The ease by which the dichloroacetyl group can be hydrolyzed prompted us to apply it to the preparation of still another degradation product of sphingolipids, namely sphingosine phosphorylcholine. The synthesis of its dihydro derivative is nearly complete.

I would like to emphasize two points which emerge from these observations. First of all, the method of synthesis of disubstituted sphingosine offers a more facile approach to unequivocal syntheses of sphingolipids. Instead of using a starting material which needs about 15 steps for its preparation, crude natural sphingosine or the more easily available synthetic dihydrosphingosine may be employed. Secondly, the use of the dichloroacetyl group for protection of the amine function may facilitate the synthesis of more complicated sphingolipids. For example, the glycosidation reaction with monosaccharides is known to give moderate or low yields. It proceeds even more sluggishly with a disaccharide. We have observed that the long chain fatty acid in the aglycon is an additional hampering factor. On the other hand it was found that an aglycon containing the dichloroacetyl instead of the fatty acid residue gives rise to considerably higher yields. Since the dichloroacetyl group is easily re-

movable and can be replaced by a fatty acid at the last stage, this method offers better prospects for a synthetic approach to ceramide polysaccharides.

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Gas-Liquid Chromatography of Glycerides

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Abstract

Available techniques are reviewed for gas-liquid chromatography of mono-, di- and triglycerides and their chemical modification products. Major emphasis is placed upon optimum separation and quantitative estimation of the recorded peaks. Nonlinear rates of temp programming are shown to be superior to linear rates for maximum resolution of complex glyceride mixtures. The use of short columns improves the recoveries of the components but may not provide a sufficient number of theoretical plates for all types of separations. The quantitative interpretation of the results is facilitated by the utilization of a flame ionization detector which gives correct weight response for the combustible carbon content of these materials.

Introduction

TRIGLYCERIDES ARE THE MAJOR components of all fats and oils of animal and vegetable origin. Diglycerides are produced by the action of phosphatidase D on phosphatides, and mono- and diglycerides are released through partial hydrolysis of triglycerides by lipases. A chromatographic fractionation of all of these lipid classes is of interest because of the information it will yield on the structure of the fats and their metabolism. In recent years it has been customary to analyze them by gas-liquid chromatography (GLC) of the constituent fatty acids as the methyl esters. It is now possible, however, to elute triglycerides including tristearin as well as the mono- and diglycerides from GLC columns without prior hydrolysis of the glyceryl ester bonds.

Separation of Triglycerides

The feasibility of GLC for triglyceride analyses has been amply demonstrated in the past and much of the initial work was reviewed by Huebner (1). The advantages of using short narrow bore columns in combination with thermally stripped SE-30 (a methyl silicone polymer obtained from the General Electric Corporation) films have since been described (2). More recently preparative systems have been developed (3) which have permitted the recovery of many of the peaks. The recovered materials have been shown to be undecomposed triglycerides by reinjection into the gas chromatograph and by enzymatic and thin-layer chromatographic methods. During the last few years gas chromatography has been applied to the fractionation of the triglyceride mixtures of vegetable oils (2), butter oil (4), their molecular distillates (5,6), adulteration mixtures (7), and their chemical (8) and enzymatic (9) reconstitution products. Essentially identical systems have permitted a direct GLC analysis of naturally occurring neutral lipid mixtures (6).

No systematic investigation of the critical parameters involved in the GLC of triglycerides has yet appeared. The following presentation illustrates the effect of some common gas chromatographic variables upon the resolution and recovery of some synthetic and natural triglyceride mixtures.

Liquid Phases

A prerequisite for high temp separations is a stable high temp liquid phase. This limits the choice to the various silicone polymers of which the most popular has been the SE-30. Thermally stripped, i.e. preconditioned, SE-30 columns were used in the first practical demonstration of the use of GLC for the separation of natural triglycerides under moderate conditions (2).

Figure 1 shows the elution patterns recorded for butterfat and a mixture of butterfat and four simple triglycerides: trilaurin (C_{36}), trimyristin (C_{42}), tripalmitin (C_{48}) and tristearin (C_{54}). The separations are based on the carbon number (mol wt) of the glycerides. A nearly complete overlap is obtained between unsaturated and saturated triglycerides of comparable chain length. This is illustrated in Figure 2 by the elution patterns given by peanut oil and a mixture of peanut oil and tristearin in a ratio of 3 to 1.

Despite acknowledged differences in selectivity with respect to many other compounds, the thermostable fluoroalkyl silicone polymer QF-1-0065 (obtained from the Dow-Corning Corporation) yielded triglyceride elution patterns similar to those recorded on the SE-30 column. A selective retention effect with this phase was not observed for triglycerides having double bonds. Figure 3 compares the gas chromatograms obtained for coconut oil on the SE-30 and the QF-1 liquid phases. The dimethylpolysiloxane gum JXR (a silicone gum produced by Applied Science Laboratories), claimed to be somewhat more stable than either SE-30 or QF-1, possessed similar triglyceride separating characteristics. Because of high velocity and easy thermal degradation the polyester phases are less satisfactory (10). It would seem that with the development of dual column systems, many other liquid phases will become available for high temp applications.

To obtain the highest possible efficiency in the separation of the high mol wt triglycerides, small samples and thin film columns should be used. Although the actual film thickness is uncertain and probably steadily changing, it has been estimated (6) that the most efficient separations are obtained with coatings at the 1% (w/w, Chromosorb W) level. Columns containing 3 to 5% of liquid phase are not recommended for work with long-chain triglycerides because of the very high