SPECIFIC LIPIDS OF MYCOBACTERIA AND RELATED ORGANISMS

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Mycobacteria and related organisms contain specific lipids not found in other natural objects. The chemical structure, localization in the cell, physiological functions, and biological activities of four groups of such lipids are considered: fatty acid derivative of carbohydrates, mainly of α,α-D-trehalose; fatty acid amides of peptides; monoglycerides with mycolic acid residues; and mycosides C – specific glycopeptidolipids of nontubercular mycobacteria. Information is given on the biological activities of synthetic analogs of the bacterial glycolipids. A separate section is devoted to the structure and biosynthesis of the specific fatty acids forming components of the lipids discussed.

Today a fairly large number of prokaryotes is known that synthesize specific lipids differing in their chemical structure from the usual membrane lipids of eukaryotes and sometimes not having even remote structural analogs among the latter. However, mycobacteria and some organisms related to them occupy a special position in this respect. They contain whole sets of lipids with unique structures. With these lipids are associated such properties of bacteria as their acid-resistance, virulence, capacity for parasitizing in the tissues of higher organisms for a long time, and capacity for living under extremely unfavorable conditions. Individual classes of such substances exhibit a powerful physiological action. It is not fortuitous that the lipids of the microorganisms mentioned are being studied intensively in various aspects in various laboratories. The specific lipids of mycobacteria and related organisms that have been described at the present time are divided according to their structural characteristics into seven main groups: 1) fatty acid derivatives of carbohydrates, mainly of α,α-D-trehalose*; 2) mannosides of phosphatidylmyoinositol; 3) peptidolipids – N-(fatty acid) derivatives of peptides; 4) mycosides C – glycosides of N-acylpeptides; 5) fatty acid esters of higher diols – phthiocerols; 6) mycosides A, B, and G – glycosides of fatty acid esters of phenolphthiocerol; and 7) glycerol mycolates. They are almost all present in cell walls. The lipids of groups 4-6 are found only in mycobacteria while the others are distributed more widely.

A number of review papers devoted to the lipid composition of mycobacteria (see, for example, the most recent [1-3]) or to individual classes of specific lipids [4-10] has been published. In the present review, the main object of discussion is formed by the results of investigations in this field during the last six years. The most interesting reports that have appeared in this period relate to the fatty-acid derivatives of carbohydrates, to peptidolipids, to the mycosides C, and to glycerol mycolates. We shall therefore limit ourselves to a discussion of just these four groups of lipids, and our main attention will be devoted to substances with established biological functions and substances with a well-defined physiological action. One of the distinguishing features of the majority of the lipids discussed below, as also of the specific lipids of other groups, is the fact that the lipophilic parts of their molecules consist completely or partially of residues of fatty acids not found in other natural materials. Some acids are also found in the free state in the bacterial cell. Basic information on specific fatty acids is given separately.

Specific Fatty Acids of Mycobacteria and Related Organisms

Of compounds of this type, the most common are the so-called mycolic acids – high-molecular-weight 2-alkyl-3-hydroxy acids (I)

*Since in the known natural trehalosolipids the trehalose has the α,α-D-configuration, the symbols for this configuration are omitted below.

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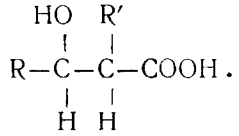
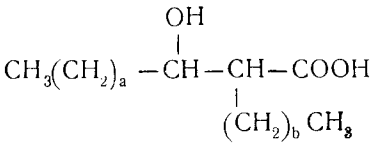


(I)

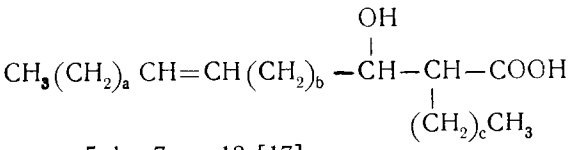
They are found in practically all mycobacteria, nocardias, corynebacteria, and representatives of other close genera that have been studied in relation to their lipid composition. The side chains (R') in the molecules of the mycolic acids have a normal structure and contain from 6 to 26 carbon atoms. The structures of the main chains (R), just like the molecular weights of the acids, are determined by the systematic positions of the organisms producing these compounds. Therefore, sometimes in order to show the molecular weight (or the number of C atoms) of an acid, to the general name "mycolic acid" is added the name of the genus for which the given molecular species is characteristic; for example, nocarbomycolic, corynomycolic, or bacterionemamycolic acid. In view of the structure given above, mycolic acids are regarded as one of the most reliable chemotaxonomic characteristics of mycobacteria and related organisms [11-13]. For the same reason, the presence of definite mycolic acids in human and animal tissues permits the conclusion that they have been infected with certain species of bacteria. Tests of this type have been proposed for the diagnosis of leprosy [14].

The mycolic acids having the lowest molecular weights and the simplest chemical structure are formed by representatives of the genera *Nocardia*, *Corynebacterium*, *Rhodococcus*, *Bacterionema*, *Gordona*, *Brevibacterium*, *Micropolyspora*, and *Arthrobacter*. These acids have unsubstituted main carbon chains (R). Below we give examples of such compounds (II-V). The mycolic acids of mycobacteria are distinguished by considerably greater structural diversity: the main chains (R) of their molecules frequently bear methyl, 1,2-methylene, and oxygen substituents. The most common are the α-mycolic acids, which include structural varieties containing no oxygen substituents in their chains (see, for example VI-IX). Acids with supplementary oxygen groupings not included among those which are characteristic of all the compounds of the class under discussion (see formula (I)) are usually found as minor components. Such an additional grouping may be a keto group (X, XI) (β-mycolic acids), a methoxy group (XII, XIII), an epoxy group (XIV), a carboxy group (XV) or an ester group (XVI) (γ-mycolic acids). All mycolic acids investigated in relation to stereochemistry have the 2R,3R configuration [36, 37]. It is interesting to direct attention to compounds (XIV): The epoxy group in the molecule of each of them corresponds to the trans configuration, while natural aliphatic epoxides with long carbon chains usually belong to the cis series.



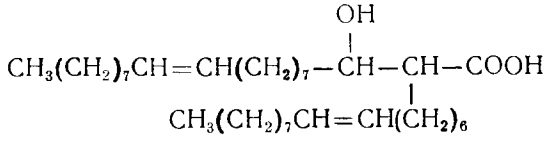
(II)

Corynebacterium diphtheriae; a=14; b=13 [17]
Bacterionema matruchotti; a=12—17; b=12—15 [18, 19]
M. paraffinicum*; a=18, 20, 22; b= 9, 11 [20]



(III)

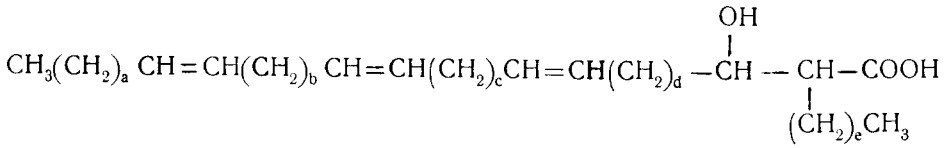
C. diphtheriae; a=5, b=7, c=13 [17]
M. paraffinicum*; a=7; b=13, 15, 17, 19; c=9, 11 [20]



(IV)

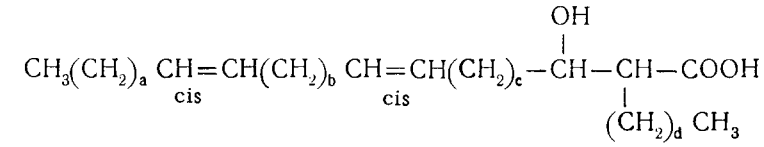
Brevibacterium thiogenitalis [21]
Corynebacterium pseudodiphtheriticum [22]

*According to recent results, this strain belongs to the genus *Rhodococcus*. "M" always denotes *Mycobacterium*.



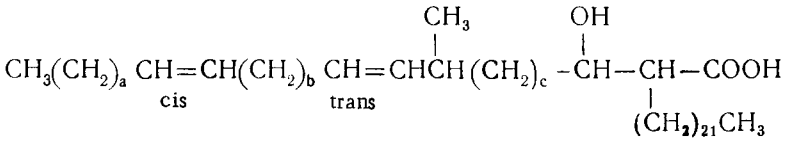
(V)

Nocardia (various species); a+b+c+d=32, 34, 36; e=13, 15 [23—25]



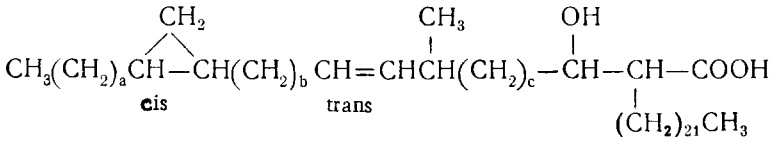
(VI)

M. phlei; a+b+c=44—50; d=21, 23 [26]
M. smegmatis; a=15, 17, 19; b=10, 12; c=15, 17, 19, 21; d=21 [27, 28]



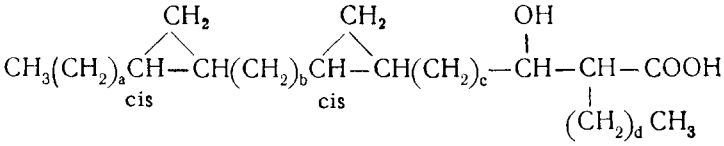
(VII)

M. smegmatis; a=15, 17, 19; b=11, 13; c=16, 18, 20 [27]



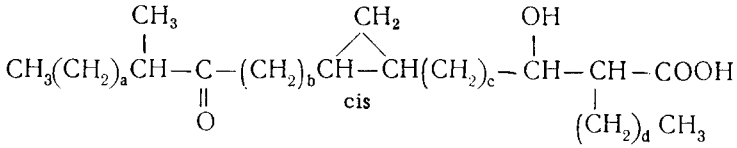
(VIII)

M. smegmatis; a+b+c=42, 44, 46; b+c=26, 28, 30 [29]



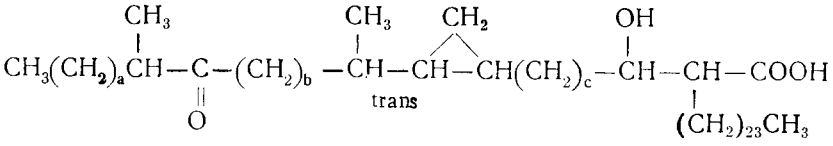
(IX)

M. tuberculosis H 37 Ra; a=17—19; b=10; c=15, 17, 19, 21; d=21, 23 [30]
M. gordonae; a+b+c=42—50; b+c=25—33; d=19, 21 [31]
M. leprae; a=17, b=14, c=17, d=19 [32]



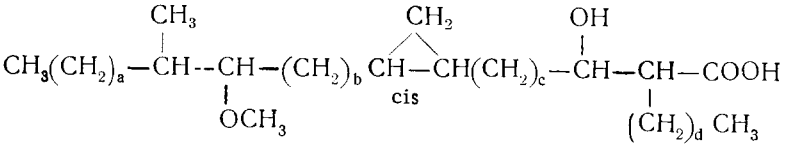
(X)

M. gordonae; a=15, 17; b+c=32, 34, 36; d=19, 21 [31]
M. leprae; a+b+c=52; d=19 [32]



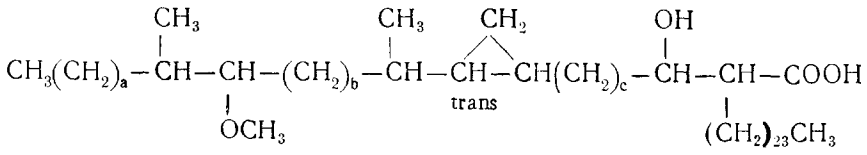
(XI)

M. microti; a+b+c=48, 50, 52 [33]



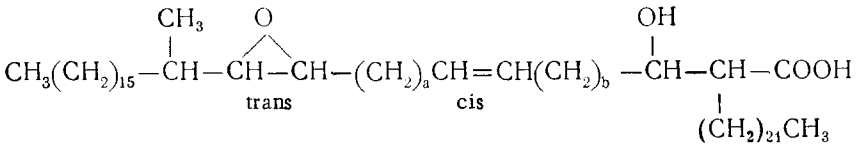
(XII)

M. gordonae; a+b+c=44—51; a+b=29—37; d=19, 21 [31]
M. microti; a=15, 17; b+c=27—37; d=23 [33]

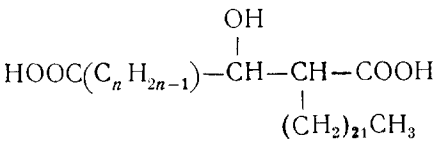


(XIII)

M. microti; a = 15, 17; b + c = 26—36 [33]
M. tuberculosis; a = 17, b = 16, c = 16 [11]

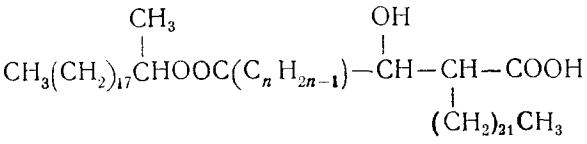


M. farcinogenes, M. senegalense [34], M. fortuitum [34, 35]; $a+b=32$ (XIV)



(XV)

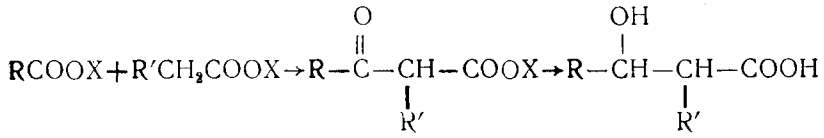
M. paratuberculosis [36], M. phlei [26, 37]; n = 34—39



(XVI)

M. phlei; n = 34—39 [26, 37]

As early as 1960, it was shown [38] that the biosynthesis of the C_{32}-corynomycolic acid in *Corynebacterium diptheriae* can be performed by the condensation of two molecules of palmitic acid in accordance with scheme 1.



(I)

R and R' are alkyl groups; concerning the possible nature of "X", see below.

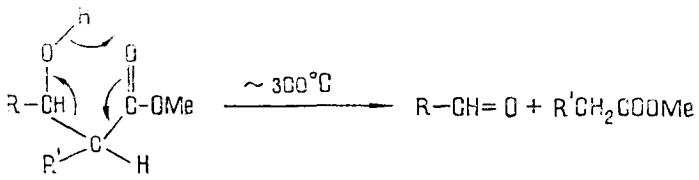
Scheme 1

It is most likely that other comparatively low-molecular-weight mycolic acids (for example, II-IV) are formed by the same route. However, hitherto the way in which the molecules of mycolid acids with complex structures that are present in mycobacteria are built up has not been definitively elucidated. The synthesis of such structures must obviously consist of several stages including the elongation of the carbon chain (R) and the introduction of double bonds and of methyl, methylene, and oxygen substituents. At present, it is known only that in the biosynthesis of these compounds in *M. smegmatis* n-tetracosanoic acid is used as the precursor of the side chain (R'), and S-adenosylmethionine acts as the donor of the C- and O-methyl and the exomethylene groups [10]. It has also been established [39] that γ-mycolic acids (XVI) are formed from β-mycolic acids (X, XI) as the result of an oxidation analogous to the Baeyer-Villiger reaction.

From the point of view of the problem under discussion, a series of papers [40-43] devoted to the isolation from *M. tuberculosis* H 37 Ra of C_{16}-C_{56} saturated and unsaturated fatty acids is of interest. Among these acids have been found, in particular, C_{34}-C_{56} acids with one and two cis-cyclopropane groupings; their structure coincides with the structure of the main chains of the α-mycolic acids synthesized by the same organisms. The authors concerned suggest that the fatty acids that they isolated are biogenetic precursors of these α-mycolic

acids. If this is actually the case, it must be concluded that in the biosynthesis of the α-mycolic acids the main carbon chain (R) is created first, after which follows condensation with a low-molecular-weight fatty acid such as n-tetracosanoic acid, and the reduction of the keto group, as illustrated in scheme 1. A detailed hypothetical scheme of biosynthesis has been given in [44, 45]. C_{49}-C_{58} fatty alcohols structurally related to the main chains of α-mycolic acids have also been detected in *M. tuberculosis* H 37 Ra [46]. Although the authors concerned [46] consider these alcohols to be products of the catabolism of the α-mycolic acids, their participation in the synthesis (or resynthesis) of the latter probably cannot be excluded.

The determination of the structures of the mycolic acids is one of the main tasks in the study of many aspects connected with mycobacteria and related organisms. We have already mentioned that the structures of these compounds form a reliable chemotaxonomic criterion and have reported the possibility of the use of the analysis of tissues for the presence of mycolic acids for the purposes of disease diagnosis. The identification of the mycolic acids is an important step in interpreting the structures of many specific lipids of the bacteria under discussion. We come up against the same acids in the investigation of the structures and functions of murein-mycoloylarabinogalactan — the structural basis of the cell wall of the mycobacteria [2, 47, 48]. Methods for the analysis, especially the routine analysis, of mycolic acids, are therefore being constantly improved.



Ia

Scheme 2

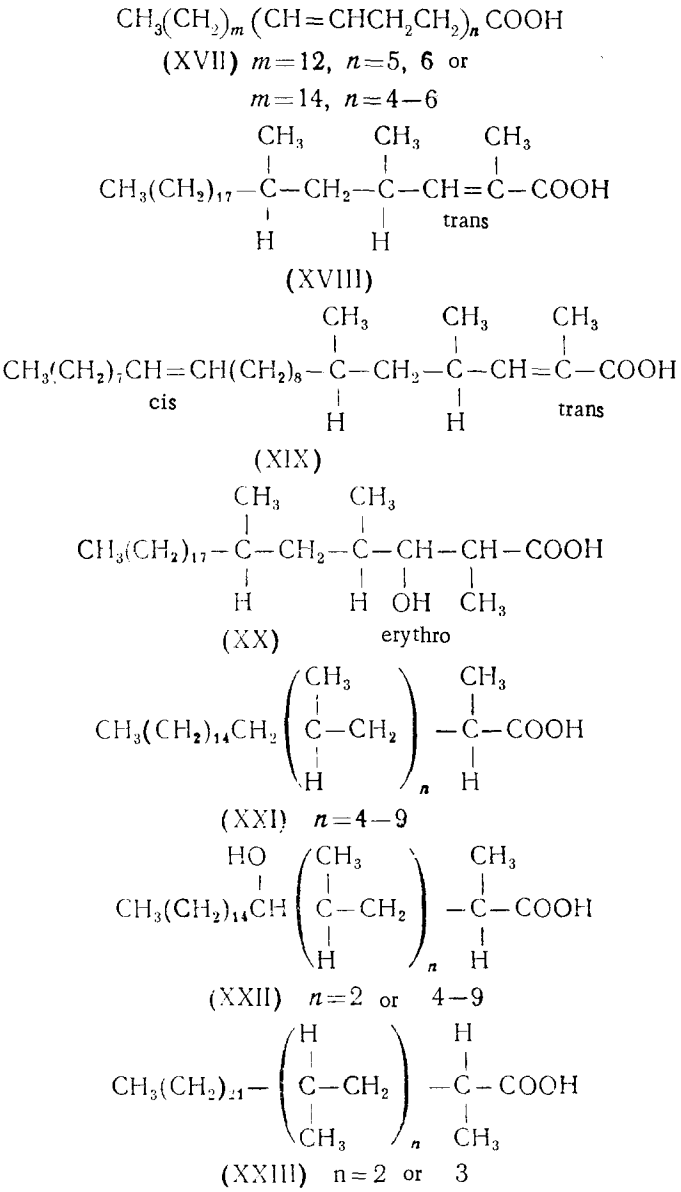
The classical method [49, 50] is based on the capacity of mycolic acid esters (Ia) for undergoing the pyrolytic decomposition illustrated in scheme 2. The pyrolysis products are identified by GLC, pyrolysis and gas-chromatographic analysis being combined, since the former takes place quantitatively in the injector of the chromatograph [50]. In spite of its simplicity, the method is inconvenient — it does not permit the unambiguous identification of an acid of complex structure and, all the more, of an acid of a previously unknown type. Even in the case of simple substances, the investigator must have available a set of standard compounds, and, moreover, it completely eliminates the possibility of the determination of the molecular-species composition of mycolic acid fractions, which is fairly frequently necessary. Nevertheless, in view of its simplicity and efficacy the classical method may prove useful in the primary screening of a large series of bacterial cultures in taxonomic investigations (see, for example, [51]).

Modern analytical methods are based on combinations of the GLC of volatile derivatives of the mycolic acids (usually the trimethylsilyl derivatives of the methyl esters) with mass spectrometry [18, 26, 52-55], chemical-ionization mass spectrometry being extremely effective [55]. But here, as well, limitations exist: In the GLC of the higher members of the series it is impossible to separate molecular species differing by the configurations and positions of the double bonds and cyclopropane groupings, and frequently even compounds with different degrees of unsaturation are not separated. The most universal and informative method consists in the fractionation of mixtures of mycolic acids with the aid of high-performance liquid chromatography followed by spectrometric (mass spectrometry, IR and NMR spectroscopy) identification of the individual substances isolated [30, 56-58]. A combination of liquid chromatography with mass spectrometry permits the determination of the composition of mycolic acids in samples containing only 10^9-10^{10} bacterial cells [58]. However, the performance of this type of analysis is technically complicated and laborious and is connected with a considerable consumption of time, which makes it unsuitable for routine determinations.

We must also mention simple and rapid methods for estimating the fractional compositions of cell mycolic acids with the aid of TLC, which provides the possibility of distinguishing some species of mycobacteria producing them [59-61].

There are grounds for assuming that the mycolic acids play an extremely important role in the life of mycobacteria — the target for the antitubercular drug isoniazid (isonicotinic acid hydrazide) is the biosynthesis of just these substances [44, 45]. Isoniazid acts similarly on corynebacteria, suppressing their growth *in vitro* [63].

In addition to mycolic acids, specific fatty acids of other types are found in the mycobacteria, but their distribution is limited to one or two classes of lipids synthesized by narrow groups of closely related froms. Such are phleic (XVII), mycolipenic (phthienic) XVIII)*, mycolipodienic (XIX)*, mycolipanolic (XX)*, phthioceranic (XXI), hydroxyphthioceranic (XXII), mycocerasinic (mycoceranic) (XXIII), and mycosanic (XXIV) acids (see the reviews [5, 10, 12]). The latter have recently been detected as components of an unidentified glycolipid in *M. aurum*. Its main fatty acid components have been characterized as 2-L,4-L-dimethyleicosanoic and 2 L,4-L-dimethyl-11- and -14-eicosenoic acids. A lower homolog — 2,4-dimethyltetradecanoic acid — has been found in *M. gordonae* [64]; 2-methyltetradecanoic acid is present in the lipids of the same organism. From the point of view of the biosynthesis of the mycolic acids, the 2-alkyl-3-keto acids (XXV) isolated from *Rhodococcus erythropolis*, where they are present in the free state, are of interest [65]. These compounds may be the direct precursors of the corynomycolic acids (see scheme 1). Similar keto acids are components of the glycolipids produced by *Corynebacterium diphtheriae* (see below).



(XVII) $m=12$, $n=5$, 6 or $m=14$, $n=4-6$

(XVIII)

(XIX)

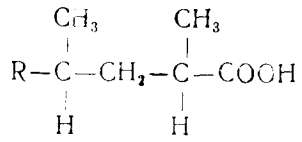
(XX) erythro

(XXI) $n=4-9$

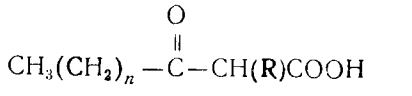
(XXII) $n=2$ or $4-9$

(XXIII) n = 2 or 3

*Structures of the main components of the fatty acid fractions are given.



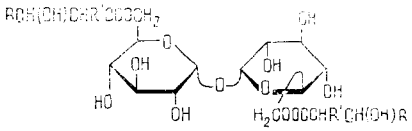
(XXIV) R=n-$C_{16}H_{33}$, n-$C_{18}H_{37}$, Δ^7-$C_{16}H_{31}$, Δ^{10}-$C_{16}H_{31}$ or κ-$C_{10}H_{21}$



(XXV) n=15–24, R=C_8H_{17}–$C_{13}H_{21}$ or C_8H_{15}–$C_{13}H_{25}$ (cis double bond)

Fatty Acid Derivatives of Carbohydrates

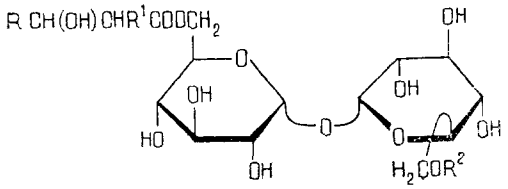
Fatty acid derivatives of carbohydrates have been studied far better than other groups of specific lipids, and among these derivatives there have been detailed investigation in all respects, while trehalose 6,6'-dimycolates (XXVI) are being studied intensively at the present time (see the reviews [3, 5, 10]). They are frequently called the "cord factor." This name



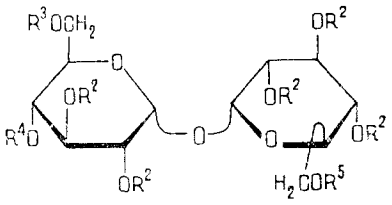
XXVI

was given to them by Bloch [66], who showed that on cultivation *in vitro* the tubercular bacilli form "cords" precisely as the result of the presence of trehalose mycolates on the surface of the cells. The trehalose dimycolates (XXVI) have been found in all mycobacteria and in the overwhelming majority of related organisms the lipids of which have been studied to a sufficient degree. A list of the strains from which the cord factor has been isolated in the pure form and has been structurally characterized can be found in the reviews [3, 5, 8]. In recent years, trehalose dimycolates have also been detected in representatives of the genus *Rhodococcus* [20, 65, 67]. In the lipids of many cultures the dimycolates (XXVI) are accompanied by trehalose 6-monomycolates (lyso cord factor) (XXVII) [8, 68-70]. A number of strains produce low-molecular-weight analogs of the cord factor. The 6'-O-acetate of the lyso cord factor (XXVIII) has been detected in *M. tuberculosis* (Aoyama B) [71]. Trehalose acylated at the primary hydroxy groups by low-molecular-weight unhydroxylated fatty acids (XXIX), mainly palmitic, has been isolated from the cells of *M. paraffinicum* [72], *M. fortuitum* (*M. minetti*) [73], and *Micromonospora* (stain Sp F3) [74]. In the last two organisms diacyltrehaloses (XXX) with an unsymmetrical arrangement of the fatty-acid residues have been identified, and in *M. paraffinicum* an analog of the cord factor (XXXI) has been found in the molecule of which one of the primary hydroxy groups of the disaccharide has been acylated by a mycolic acid and the other by a normal unhydroxylated acid (mainly $C_{16:0}$ and $C_{14:0}$) [75]. *Corynebacterium diphtheriae* synthesizes, together with the cord factor, 6,6'-diacyltrehaloses of two types [76, 77]. One of them (XXXII) contains as the acyl fragments C_{24}–C_{32} 2-alkyl-3-keto acids of the type of (XXV), and in the other glycolipid (XXXIII) one of the keto acids mentioned and one residue of a corynomycolic acid of type (II) or (III) are attached to trehalose. (See formula, top of following page.)

The group of specific lipids under discussion also includes trehalose derivatives containing three and more fatty acid residues. These include the esters of phleic acids (XVII) and trehalose that have been found in *M. phlei* and *M. smegmatis* [78]. Fractions of these glycolipids consist of polyesters with different number of phleoyl residues; among them the octaphleoate (XXXIV) predominates, and it is also the most stable component of the fraction. The other phleates decompose extremely rapidly under the usual laboratory conditions; for this reason, in particular, their structure has not yet been established. The subgroup of polyacyltrehaloses includes substances with a strongly acidic nature. Of these, the most interesting



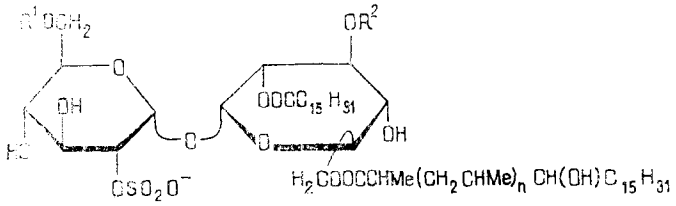
(XXVII) $R^2 = H$; (XXVIII) $R^2 = Ac$; (XXIX) $R^2 = COC_{13}H_{27}$ or $COC_{15}H_{31}$; (XXX) $R^2 = COCHR'COR$



(XXXI) $R^2 = R^4 = H$, R^3 и $R^5 - COC_{13}H_{27}$, $COC_{15}H_{31}$ или $COC_{17}H_{35}$; (XXXII) $R^2 = R^5 = H$, R^3 и $R^4 - COC_{15}H_{31}$ или $COC_{17}H_{35}$; (XXXIII) $R^2 = R^4 = H$, R^3 и $R^5 - COCHR'COR$; (XXXIV) R^2, R^3, R^4 и R^5 — phleoyl

(see formula XVII)

Everywhere R and R' = alkyl

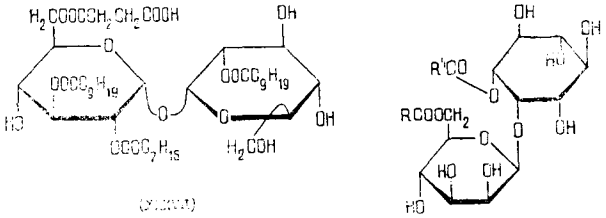


(XXXV) R^1 — hydroxyphthioceranoyl (see formula XXII), R^2 — phthioceranoyl (see formula XXI); (XXXVI) $R^1 = H$, R^2 — hydroxypthioceranoyl

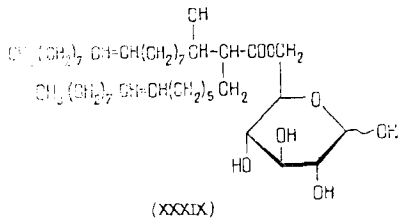
according to the published results of biochemical and biological investigations are the so-called sulfolipids I, II and III [79-81] produced by virulent strains of *M. tuberculosis*. The main component of the sulfolipids-I fraction has been characterized as 2-palmitoyl-3-phthioceranoyl-6,6'-bis(hydroxyphthioceranoyl)trehalose 2'-sulfate (XXXV) [80]. The minor components differ from the main component (XXXV) by the presence of a stearic, in addition to a palmitic, acid residue and (or) by the composition of the hydroxyphthioceranic acids. Sulfolipids-II and -III are triacyl derivatives of trehalose 2'-sulfate and contain no phthioceranic acids [79, 81]. Sulfolipid-III has been assigned the structure of 2-palmitoyl-3,6-bis(hydroxyphthioceranoyl)trehalose 2'-sulfate (XXXVI) [81]. The positions of the acyl residues in the sulfolipid-II molecule remain unknown at the present time.

An acid trehalosolipid of a different nature (XXVII) has been isolated from the paraffin-oxidizing bacterium *M.paraffinicum* [75, 82]. The acidic nature of this substance is due to the presence in its molecule of a monoesterified succinic acid residue. In contrast to the glycolipids described above, the lipophilic moiety of the molecule of (XXXVII) consists only of residues of short-chain fatty acids — n-octanoic and n-decanoic acids. (Formula, top, following page.)

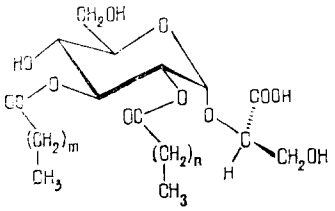
Fatty acid derivatives of carbohydrates other than trehalose have been detected in a few cultures of mycobacteria and related organisms. The diacyl derivatives of α-D-mannopyranosyl-myoinositol (XXXVIII) synthesized by *Propionibacterium shermanii* can be regarded as remote structural analogs of the cord factor [83, 84]. The predominating molecular species of this glycolipid fraction is 1(3)-O-pentadecanoyl-2-O-(6-O-pentadecanoyl-α-D-mannopyranosyl)-sn-myoinoisitol XXXVIII, R = $C_{16}H_{33}$, R' = $C_{14}H_{29}$). The opinion exists that compounds (XXXVIII) are



(XXXVII)



(XXXIX)



(XL) m and n = 12, 14, and 16

intermediate products of the metabolism of phosphatidylmyoinositol mannoside (see a review on these glycophospholipids [85]). A mutant of *Brevibacterium thiogenitalis* requiring oleic acid for growth synthesizes a 6-mycoloylglucose (XXXIX) [21], which is not retained in the cells and passes into the culture medium. A glycolipid of a completely new type has been isolated from *Nocardia caviae* [86]. The structure of 2-O-(2,3-Di-O-acyl-α-D-glucopyranosyl)-D-glyceric acid (XL) has been assigned to it [87]. It must be mentioned that natural compounds containing glyceric acid residues are found extremely rarely, and lipids of this type have not previously been known.

Some organisms form acyl sugars when they grow on media with high concentrations of these sugars. Thus, a 6-O-mycoloylglucose, analogous to (XXXIX), forms a considerable part of the cell lipids in *M. smegmatis* and *C. diphtheriae* when they grow in the presence of a large amount of glucose [88]. Individual strains, when cultivated on a medium containing fructose as the sole source of carbon, produce mono- and diacylfructoses [89]: *Arthrobacter paraffineus* synthesizes fatty acid derivatives of sucrose when grown on a medium including this disaccharide [90]. The structures of the acyldisaccharides mentioned have not yet been definitively established.

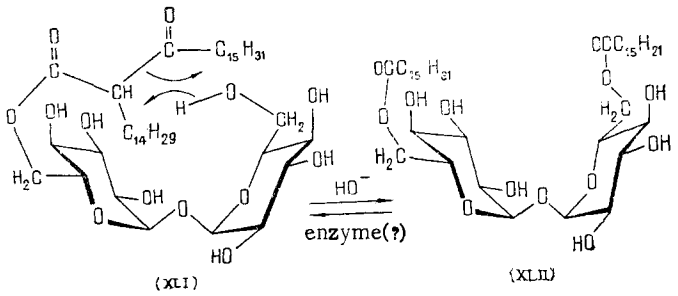
Many authors also assign 5-O-mycoloyl-D-arabinofuranose and 2-O-(5-O-mycoloyl-α-D-arabinofuranosyl)-D-arabinofuranose to the specific lipids of mycobacteria and related organisms (see, for example, [2, 9, 48]); however, strictly speaking, these substances cannot be regarded as native lipids since in the cells they are covalently bound to the arabinogalactan-murein complex, and destructive methods must be used for their isolation. We shall refer to the arabinomycolates in a discussion of the biological activity of the cord factor, since the physiological action of the former coincides partially with that characteristic of the trehalose mycolates.

The glycolipids described above exhaust the known structural types of fatty acid derivatives of carbohydrates characteristic for mycobacteria and related organisms. Let us dwell on the most important biosynthetic and biological aspects connected with the lipids that have been discussed.

A simple comparison of the structural formulas of the trehalose di- and monomycolates (XXVI and XXVII) leads to the idea that the latter may serve as biogenetic precursors of the former. Nevertheless, this has been successfully demonstrated only recently. It has been established that a cell-free preparation of *M. smegmatis* contains an enzyme (an acyl transferase) transferring a mycolic acid residue to exogeneous trehalose monomycolate (XXVII) and that in the same preparation the mycolic acids are present in an active form (it is assumed that

they are attached to a protein by a sulfide bond) [91]. It is striking that the antimycobacterial agent ethambutol, which inhibits the biosynthesis of trehalose mono- and dimycolates [92], does not affect the activity of the acyl transferase that has been found [91].

A number of facts indicate that trehalose monomycolates may play the role of an intermediate stage in the metabolism of the mycolic acids. When radioactively labeled palmitic acid was incubated with a cell-free preparation of *C. diphtheriae*, labeled 6-O-(2-tetradecyl-3-keto-octadecanoyl) trehalose (XLI) was formed [93, 94], i.e., if we refer to scheme 1, the function "X" in this case is fulfilled by a trehalose residue. The reduction of the keto ester (XLI) to trehalose monomycolate (XXVII) would simultaneously complete the construction of the corynomycolic acid molecule. If we take into account the high rate of exchange of mycoloyl residues in the monomycolates (XXVII) [92], this biosynthetic route to the simplest mycolic acids appears completely feasible. On the other hand, the keto ester (XLI) readily undergoes an intramolecular rearrangement under weakly alkaline conditions and is converted into 6,6'-di-O-palmitoyltrehalose (XLII) [95] (scheme 3).



Scheme 3

No similar breakdown of a ketoacyl residue takes place when the hydroxy group at $C_{(6')}$ is substituted or is absent [95]. Thus, the trehalose fragment of the (XLI) molecule acts as a kind of catalyst which, adopting the corresponding conformation, brings the keto group of the 3-ketoacyl residue close to the free OH group at $C_{(6')}$ and thereby promotes the cleavage of this residue. A process takes place that is the reverse of the one which is illustrated in scheme 1, and a trehalose residue once again takes on the role of "X". It may be assumed that in bacterial cells the degradation of the mycolic acids takes place through the stage of trehalose monomycolates, which are oxidized to keto esters of type (XLI) and the latter undergo rearrangement under the action of a definite enzyme into the 6,6'-diacyltrehalose (XXIX), which has been detected in the lipids of some bacteria (see above). It is considered that the reverse process, i.e., the synthesis of the monomycolates (XXVII) from the 6,6'-diacyltrehaloses (XXIX) via the stage of keto esters of types (XLI) is equally possible [9]. A hypothesis has been proposed of the existence of a parallel route of the biosynthesis and decomposition of the mycolic acids according to which the construction of their molecules is the result of the addition of a fatty aldehyde to an activated fatty acid [38, 76] and decomposition takes place via a stage of ketoacyltrehaloses such as has been described above (XXXII and XXXIII) [76].

The hypothesis has been expressed that trehalose monomycolates function as transporters of mycolic acids and, in particular, transfer them to the arabinogalactan of the cell wall [9]. An experimental confirmation of the participation of 6'-acetyl-6-mycoloyltrehalose (XXVIII) in this process has been obtained [71].

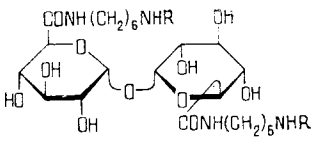
Even before the structure of the cord factor had been established, facts were known that indicated its localization on the surface of the cell wall [96]: In the first place, the treatment of the cells of mycobacteria with petroleum ether freed them almost completely from the glycolipid (XXVI), but the cells retained viability; in the second place, cultures of mycobacteria are capable of growing on media with a high concentration of Tween 80, while under these conditions the cord factor does not remain in the cells but is liberated into the medium. A bond of trehalose dimycolate with the cell wall was also shown by the results of a direct analysis of a purified fraction of *M. avium* cell walls [5]. In a similar manner to the cord factor, the extraction of a culture of *M. phlei* with hexane eliminated trehalose phleoates but the capacity of the culture for growth was not lost after this. An investigation of subcellular fragments of *M. phlei* showed that the phleoates were completely bound to a fraction

containing diaminopimelic acid, which, as is well known, is a cell wall marker. Consequently, these glycolipids, as well, were localized on the surface of the cell wall. This may also be considered as the location of the sulfolipids, since they are extracted under mild conditions by the brief treatment of the cells with hexane containing only 0.05% of decylamine [97]. Furthermore, the presence of sulfolipids explains the fixation of the dye Neutral Red by the cells of virulent strains of *M. tuberculosis* [97].

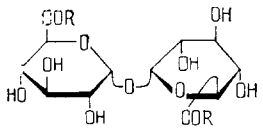
Various hypothesis (some of which are given in the review [5]) have been put forward in favor of a possible physiological role of fatty acid derivatives of trehalose, but not one of them has received more or less convincing experimental confirmation. Nevertheless, the results of the experiments performed have permitted a number of hypotheses to be rejected. Thus, it has been established that in *M. smegmatis* the rate of exchange of trehalose mycolates is 13 times greater than the rate of their "pure" biosynthesis [98]. Hence, these lipids can fulfill neither a structural nor a reserve function. The latter cannot be assigned to trehalose phleoates, either, since they, unlike the simplest derivatives of ordinary fatty acids (triglycerides, waxes, etc.), are not utilized by bacteria when a source of carbon is in short supply [78]. Phleoates are not among the obligate lipids of mycobacteria — they are formed under certain conditions by saprophytic organisms — *M. phlei* and *M. smegmatis* — but are absent from *M. tuberculosis* and *M. bovis* BCG [78]. These lipids probably play a fundamental role in the aggregation of bacterial cells. It has been observed that they are not synthesized in the growth of *M. phlei* in the dispersed state but, conversely, the synthesis of trehalose phleoates is intensified under conditions favorable for the appearance of cell associates [78].

In the 50s, the cord factor attracted the attention of research workers not only by the unusual nature of its structure but also by its toxicity for laboratory animals. A detailed study of the toxic effect of purified preparations of cord factor and its analogs was made by Kato. He established that the LD_{50} value of trehalose dimycolates for mice on intraperitoneal administration amounted to from 50 to 120 μg per animal, depending on the structure of the mycolic acids [99, 100]. Glycolipids with residues of low-molecular-weight mycolic acids were the least toxic. The toxicity of the trehalose monomycolates (XXVII) [101] and that of the diacyltrehaloses (XXIX) and (XLII) were even lower — the LD_{50} values were 387 and 200 μg, respectively, in mice. All the trehalosolipids mentioned exhibit a toxic effect only on administration to animals in mineral oil or in an aqueous oil emulsion, and its cause is the capacity of these lipids for uncoupling oxidative phosphorylation and the respiratory chain in the mitochondria, their action on the system of oxidative phosphorylation being directed selectively to the cytochrome b → cytochrome c_1 section [99].

To explain the dependence of the toxic effect of the trehalosolipids on features of their structure, various lipid derivatives of carbohydrates and of other polyols have been synthesized: a sucrose 6,6'-dimycolate [102], 6-O-mycolates of methyl α- and β-glucopyranosides, of methyl α-galactopyranoside, of methyl α-mannopyranoside, and of methyl α-allopyranoside [102, 103], monomycolates of glycerol and of sorbitol [104], 6-O-acyl derivatives of methyl glucopyranosides, and 6,6'-diacyltrehaloses with various fatty acid residues (105-110), and also so-called "pseudo cord factors" — compounds (XLIII and XLIV) with a different type of bond between the hydrophilic and the lipophilic fragments of the molecule [111].



(XLIII) $R = CO(CH_2)_3C_6H_4COC_{15}H_{33}$ or 3-mycoloyl



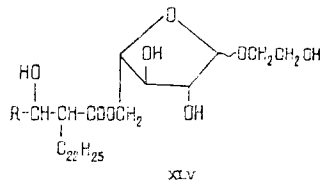
(XLIV) $R = NHC_{10}H_{21}$, $NHC_{18}H_{37}$, $N(C_{16}H_{37})_2$ or $O(CH_2)_4C_6H_4OC_{15}H_{33}$

The study of the action of aqueous dispersions of the substances mentioned on isolated mitochondria has shown [109] that oxidative phosphorylation is inhibited only by glycolipids containing in their structure the 6-O-acylglucopyranose grouping that is present in the molecule of the cord factor (XXVI), i.e., derivatives of trehalose, sucrose, and of glucopyranosides. The LD_{50} values of these compounds (in the form of a water-oil emulsion) for mice lay between the values corresponding to trehalose di- and monomycolates. Galactoside, mannoside, and alloside mycolates were completely inactive *in vitro* and nontoxic for the animals.

Thus, the configurations of the $C_{(2)}$, $C_{(3)}$, and $C_{(4)}$ atoms of the carbohydrate residue of the glycolipid have decisive value for the manifestation of the type of activity under consideration. Hydroxy groups at these C atoms should be free — acetylated cord factor does not cause either morphological or functional changes in animals [112]. A weak inhibiting action on oxidative phosphorylation was observed *in vitro* for mycoloylsorbitol, the stereostructure of which is identical with that of glucose, but no effect was detected in experiments with the analogous glycerol derivatives [104]. Activity with respect to the respiratory chain of mitochondria was exhibited only by disaccharide mycolates [109]. An investigation of the interaction of a fluorescent analog of the cord factor — methyl 6-O-[12-(9-anthroyl)stearoyl]-α-D-glucopyranoside — with mitochondria showed that such substances are irreversibly bound to the mitochondrial membrane [113].

Modifications of the fatty acid residues — a change in the configuration of the $C_{(3)}$ asymmetric center of the mycolic acids, the elimination of the hydroxy group, and even the replacement of the mycolic acids by normal unhydroxylated fatty acids with more than 20 carbon atoms — do not substantially affect the capacity of trehalose and glucoside derivatives for suppressing oxidative phosphorylation [107, 108]. A change in the nature of the bond between the lipophilic and carbohydrate fragments of the molecule has no appreciable effect, either (see the pseudo cord factors) [111]. However, activity in relation to the respiration of the mitochondria falls with a decrease in the lengths of the chains of the fatty acid residues [109]. The cord factor acts practically identically on mitochondria isolated from the corresponding organs of mice and rats, but trehalose 6,6'-dimycolates are nontoxic for rats [114] and for guinea-pigs [115]. It is assumed that the cell membranes of these animals are impermeable for these glycolipids [114]. Such an interpretation is difficult to accept without experimental confirmation.

In vitro, the "natural" mycolate of arabinofuranosylarabinofuranose inhibits the oxidative phosphorylation system of mitochondria to the same degree as the cord factor [109]. Arabinofuranose mycolate exhibits no effect whatever in this respect, but its 2-hydroxyethyl glycoside (XLV) acts in the same way as the cord factor of arabinobiose mycolate [109]; it is still impossible to give any explanation whatever of such a sharp difference in the properties of substances with similar structures.



XLV

In the course of the investigations described above, an extremely important fact was revealed. It was found that the activity of the cord factor and its analogs *in vitro* depend greatly on the phase state of the lipid vesicles in the aqueous dispersions used for the trials. It was considerably higher when the vesicles were present in the "fluid state" [108, 116]. Therefore, when the activities of one and the same glycolipid are determined under even very close, but not identical, temperature conditions, it is possible to obtain sharply differing results if these conditions are selected on different sides of the phase-transition temperature.

One of the most important and interesting properties of the trehalose 6,6'-dimycolates is their immunoreactivity [117, 118]. The cord factor injected into mice or rabbits in the form of a complex with methylated bovine serum albumin (MBSA) causes the formation of antibodies [119]. When the antiserum obtained from the immunized animals is mixed with an aqueous emulsion of the cord factor, a precipitation reaction is observed and a considerable amount of glycolipid is bound with the precipitate. In rabbit serum, the precipitated antibodies have been found in the 19S macroglobulin fraction (IgM) [120]. It has been established that the antigenic determinant in the cord factor—MBSA antigenic complex is the trehalose residue. In mice immunized with this complex a resistance arises to the toxic action of the cord factor — in animals that had been given more than 1 mg of the glycolipid in mineral oil (in doses 7-10 times greater than the lethal dose) there were no pathological changes whatever in the mitochondria. The animals also exhibited a specific resistance to tuberculous infection, but they remained sensitive to infection with brucellas, salmonellas, and listerias [121]. The immun-

ization of mice with the "sucrose 6,6'-dimycolate–MBSA" or the "methyl 6-O-mycoloylglucopyranoside–MBSA" complex did not lower their sensitivity to the cord factor, which confirms the determinative role of the trehalose residue in the "cord factor–MBSA" antigenic complex [121]. The cord factor gives a positive reaction with the serum of tuberculosis or leprosy patients on enzyme-linked immunosorbent analysis (ELISA) and, consequently, can be used in the immunodiagnosis of these diseases [122].

A water-oil emulsion of the cord factor acts as an adjuvant [117, 118, 123-128]; this form of activity appears most clearly in mice and rats and to a considerably smaller degree in guinea-pigs. Many biological effects caused in animals by pathogenic and facultatively pathogenic mycobacteria are reproduced with the aid of a water-oil emulsion of the cord factor. Such effects include: the initiation of the granulomatosis reaction [128-130], an intensification of the immune response to foreign antigens [123, 125], the stimulation of macrophages [131], the activation of the alternative route of complement [132], a proliferative response of lymphocytes [133], an increase in sensitivity to bacterial infections [123, 134-136] and to parasitic protozoans [117, 137], and the inhibition of the growth of malignant neoplasms [138-146]. In relation to the last-mentioned property that the cord factor has proved to be an effective agent in the immunotherapy of malignant tumors in mice [145], guinea-pigs [139, 142], and man (see the review [131]), and, in combination with killed cells of *M. bovis* BCG, has been used for the preparation of an anticancer vaccine [138]. The antitumoral activity of the cord factor is substantially raised by the peptidoglycan of the mycobacterial cell wall [138-141]. With the aid of such a combined preparation it has been possible, for example, to achieve a considerable regression of a hepatocarcinoma in guinea pigs [140, 141], and also an 83% suppression of skin cancer and metastases in the lymph nodes of the same animals [139]. Recently, a combination of the cord factor with synthetic N-acetylmuramyl-L-alanyl-D-isoglutamine has attracted attention, its use as a therapeutic preparation having led to the complete regression of a hepatocarcinoma in guinea-pigs [117].

All the types of activity of trehalose 6,6'-dimycolate mentioned above appear when it is administered to animals in water-oil emulsion, and the quantitative expression of the biological effect depends on the size of the oil particles in the system [147, 148] and on the weight ratio of glycolipid to oil [145]. This relationship is explained by the fact that trehalose dimycolates exhibit physiological activity only when their molecules are arranged in the form of a monolayer on the surface of a hydrophobic carrier [149], the role of which in the examples described above is played by the particles of mineral oil. The function of the support may also be performed by solid polystyrene spheres with a diameter of about 6 μ [150]. In the absence of a carrier, the trehalose dimycolates readily form micelles in the aqueous phase [65] that are completely inactive [150]. The biological activity of a monolayer of the cord factor is closely connected with its capacity for sorbing fibrinogen [150], its micelles being deprived of this capacity. Some of the natural and synthetic analogs act in a similar manner to the trehalose 6,6'-dimycolates [7, 111, 117, 126, 134, 151-153].*

The sulfolipids-I, -II, and -III are nontoxic for animals [155] and, *in vivo*, cause no changes in the mitochondria. However, in isolated mitochondria the sulfolipids uncouple oxidative phosphorylation far more actively than the cord factor [155], this action being completely suppressed by the addition of serum albumin to the incubation medium. The nontoxicity of sulfolipids for animals is explained by the antagonist effect of this protein. On the combined administration of sulfolipid-I (or -III) and the cord factor, the toxicity of the latter is increased almost 9-fold [155]. An increase in toxicity is also observed when these lipids are administered by different routes – for example, the cord factor intravenously and the sulfolipids intraperitoneally. No such synergism occurs when the cord factor is replaced by a trehalose monomycolate (XXVII) or by mycolates of methyl glucosides.

Long before the isolation and identification of the sulfolipids, it was observed that the sulfate-containing lipids were present only in virulent strains, and with an increase in virulence the level of these substances in the bacterial cell rose [97]. At the same time, no correlation whatever was observed between the amount of cord factor and virulence. Later, as the result of an investigation of a large number of strains of *M. tuberculosis* belonging to various phagotypes and collected from tuberculosis patients in India, Burma, and East Africa, a statistically significant correlation was found between the level of "strongly acidic lipids," i.e., sulfolipids, and acidic phospholipids, on the one hand, and the virulence of the strains for

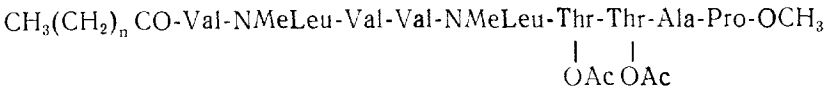
*Additional information on the biological activity of the cord factor, of its analogs, and of other mycobacterial lipids can be found in the review [154].

guinea pigs, on the other hand [156, 157]. It is true that exceptions were also found — in 22 highly virulent strains of phagotypes B and I obtained in Iran and the United Kingdom the level of "highly acidic lipids" was anomalously low [157]. Nevertheless, the participation of the sulfolipids in the pathogenesis of tuberculosis is fairly obvious.

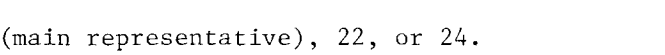
Peptidolipids

The peptidolipids, which are fatty acid amides of peptides, form one of the classes of lipids that have been little studied. They are found in bacteria and lower fungi. The structures of these compounds — particulary the peptide fragments of the molecules — are extremely diverse. The peptidolipids known at the present time contain from four to 14 amino acid residues and the peptide chains are either linear or cyclic or of mixed structure; the amino acids have both the usual L and also the unusual D configuration, both antipodes of an amino acid sometimes being present in the same molecule; and unique amino acids are not infrequently found. Characteristics of the peptidolipids of mycobacteria and related organisms is a specific fine structure.

Although the first papers on the peptidolipids of mycobacteria and nocardias appeared as early as the 60's, the number of substances of this class isolated from these microorganisms is small. Two peptidolipids have been found in mycobacteria: fortuitin (XLVI) [158], produced by *M. fortuitum*, and a lipid (XLVII) present in the cells of *M. paratuberculosis* (*M. johnei*) [159]. It can be seen from the formulas given that both lipids have simple linear peptide chains, the C-terminal amino acid being esterified, as in the overwhelming majority of compounds of the class under discussion, and the lipid fragments of the molecules being residues of normal saturated acids.

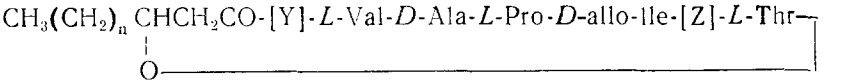


(XLVI), fortuitin; all amino acids of the L configuration; n = 18 or 20.



(XLVII) n = 16, 18, 20 (main representative), 22, or 24.

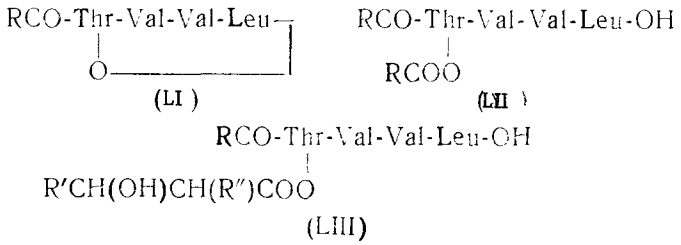
The peptidolipids (XLVIII-L) detected in *Nocardia asteroides* have more complex structures [160-163]. The main component of the peptidolipid fraction is peptidolipin NA (XLVIII) [160, 161]. This lipid and also (XLVII) are examples of the coexistence of two antipodes of an amino acid in one molecule. Minor components, Val^6-peptidolipin NA (XLIX) [162] and



(XLVIII) Y=*L*-Thr, Z=*L*-Ala, n=16; (XLIX) Y=*L*-Thr, Z=*L*-Val, n=16, 17 or 18; (L) Y= L-α-aminobutyryl Z=*L*-Ala, n=16, 17 or 18

α-aminobutyryl^1-peptidolipin NA (L) [163] differ from the main component (XLVIII) by the presence of L-valine and L-α-aminobutyric acid in place of L-alanine and L-threonine, respectively. The peptidolipins exhibit some activity against Gram-positive bacteria.

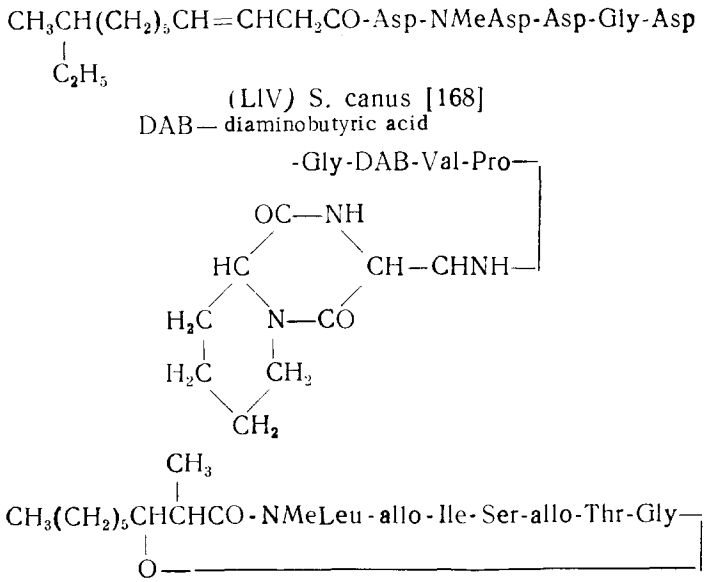
A whole family of seven peptidolipids has been isolated from the paraffin-oxidizing bacterium *M. paraffinicum* (for the systematic position of the microorganism, see the footnote above) [164-166]. The structures of the three simplest of these compounds (LI-LIII) have been established completely. They proved to be fairly unusual. In the molecule of (LI), the peptide fragment forms a ring consisting of only four amino acid residues [165]. The acidic nature of the other two lipids (LII) and (LIII) is an extremely rare phenomenon. Furthermore, these substances both contain additional fatty acid residues attached by ester bonds to the peptide moiety [166]; in lipid (LIII) this residue is that of a mycolic acid. So far, this lipid has remained the only fully characterized peptidolipid containing mycolic acid. Mycolic acid has also been detected in a peptidolipid from *Corynebacterium lepus* [167], but its structure has not been definitively established. Four unidentified peptidolipids from *M. paraffinicum* have no biogenetic link with the lipids (LI-LIII), so far as can be judged from the results



where RCO represents residues of C_{20}-C_{28} saturated and monoenoic acids; R', C_{17}-C_{19} alkyl; R'', C_{10} or C_{12} alkyl; all amino acids of the L configuration.

of a preliminary analysis [164]. Their peptide chains consist of residues of glycine, L-leucine, D-alloisoleucine, L-threonine, L-serine, L-homoserine, and D-alanine and include not less than 14 amino acid residues. Mycolic acids are attached to the peptide fragments of the molecule by ester bonds, and the serine residues are glycosylated with glucose.

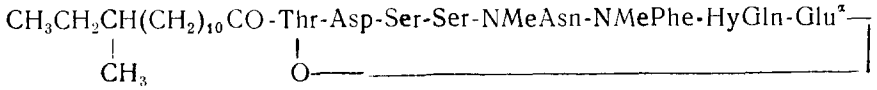
Many peptidolipids possess antibiotic activity to some degree or other. For this reason, it has been possible to detect a number of compounds of this class in streptomycetes during the screening of cultures for the presence of antibiotics. As examples of peptidolipids produced by streptomycetes we give below the structure of amphomycin (glumamycin) (LIV), globomycin (LV), and lipopeptide A (LVI) with the names of their producing agents



(LIV) S. canus [168]

DAB — diaminobutyric acid

(LV) S. halstedii, S. neohygroscopicus, S. hagronensis, Streptoverticillium cinnamoneum [169]



(LVI) S. violaceochromogenes [170]

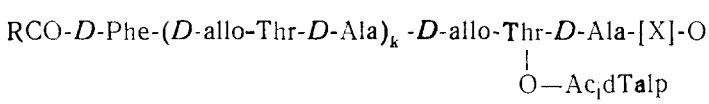
HyGln — threo-β-hydroxyglutamine

In formulas (LV) and (LVI), all the amino acids have the L configuration.

It is considered [9] that peptidolipids are synthesized by many mycobacteria and related microorganisms, but in the study of the lipid composition of bacteria they disappeared from the field of view of research workers since the majority of these substances (even substances with well-defined lipophilic properties, such as globomycin (LV) are not retained in the cells and are liberated into the medium. The lipids of the culture medium were analyzed only in individual cases. It is assumed [9] that an analogy exists in the biosynthetic routes of the peptidolipids and the peptide antibiotics such as gramicidin S and tyrocidine.

Mycosides C

Mycosides C are specific glycolipids of nontubercular or, as they are also called, atypical mycobacteria. In contrast to the peptidolipids (XLVI-LVI) described above, the lipopeptide chains of the mycosides C are constructed by the general scheme: RCO-D-Phe-D-Allo-Thr-D-Ala)$_n$-X, where the lipid moiety (RCO) is the residue of a normal saturated or monoenoic acid with 24 or more carbon atoms containing, as a rule, a hydroxy or methoxy group at $C_{(3)}$. The peptide chain most frequently contains a single (D-Allo-Thr-D-Ala) dipeptide unit, i.e., n = 1. Mycosides with n = 2 and 3 have been isolated, but the structure of the lipid with n = 3 has not been fully demonstrated. The function of the terminal unit of the peptide chains usually bears a residue of the amino alcohol L-alaninol, but in the mycoside C from *M. butyricum* (see Table 1) this unit is N,O-dimethyl-L-serine [171] and in the mycoside fraction from *Mycobacterium sp.* 1217 one of the components contains as "X" an ethanolamine residue [172]. The component "X" is linked by a glycosidic bond to partially O-methylated L-rhamnose. The hydroxy group of the threonine residue (in the mycosides with n > 1, the residue closest to "X") is also glycosylated and, depending on whether a monosaccharide (partially acetylated 6-deoxy-L-talopyranose) or an oligosaccharide is attached to it, the mycosides C are subdivided into nonpolar and polar [173, 174]. Mycoside C'_{378} isolated from the strain *M. farcinogenes* 378, the molecule of which has only one monosaccharide residue at the L-alaninol, must also obviously be assigned to the nonpolar group [175]. The mycoside fractions of some strains contain molecular species differing by the degree of methylation of the L-rhamnose. Below we give the general formula (LVII) of the nonpolar mycosides C, while Table 1 shows the mycobacteria synthesizing these lipids, the arbitrary designation of the mycosides, and also details of their structure: the nature of "R" in the fatty acid residue, the number of dipeptide units (k) apart from the glycosylated one, the nature of "X," and the structure of the O-methylated L-rhamnopyranose (M — 3-O-methyl-L-rhamnopyranose; D — 3,4-di-O-methyl-L-rhamnopyranose; T — 2,3,4-tri-O-methyl-L-rhamnopyranose).



(LVII) k = 0, 1, or 2; l = 1 or 2; m = 1, 2, or 3.

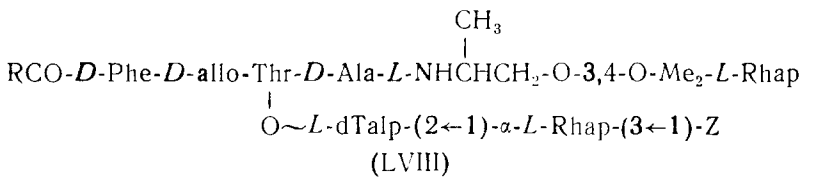
The polar mycosides C have attracted the attention of research workers comparatively recently in connection with the results of an immunological and morphological study of the atypical mycobacteria, which form smooth colonies. In the microorganisms mentioned species- and type-specific antigens (Schaefer antigens) uncharacteristic of the "typical" mycobacteria have been found [183]. This fact has served as the basis for the development of a seroagglutination method of identifying and classifying the atypical mycobacteria [183]. The use of the method for the immunoanalysis of a wide range of the latter has permitted the discovery of a distinct serological group of mycobacteria consisting of 31 related but strictly individualized serotypes [184, 185]. Today it is called the *M. avium—M. intracellulare—M. scrofulaceum* (MAIS) serocomplex [184, 185].

Chemical analysis of the antigenic material of representatives of the complex has shown that the substances composing it possess lipophilic properties and that their structures include the same linear N-acylpeptidoglycoside chain that is the basis of the molecules of the nonpolar mycosides, but the threonine residue of the chain is bound to an oligosaccharide [174, 186]. The further investigation of these antigens [187, 188] has led to the conclusion that they all have the general formula (LVIII) and differ only by the nature of the carbohydrate (Z) attached to the α-L-rhamnose residue. The "Z" fragment is strictly specific for each serotype. At the present time, the structures of the polar mycosides isolated from the cells of serotypes 8, 9, and 25 (LVIIIa, b, and c) have been established, and only the anomeric configuration of the 6-deoxytalose residue attached directly to the peptide chain has not been determined [188]. Facts are known indicating that in the analogous lipids of other serotypes the "Z" fragment also consists of not more than two carbohydrate residues (see [188]). Thus, the serological specificity of the antigens under discussion is coded by one or two monosaccharides located at the nonreducing end of the oligosaccharide chain. In this respect, the polar mycosides of the atypical mycobacteria differ from the O-antigens of the enterobacteria and, rather, are comparable with the group glycosphingolipids of the blood [189].

TABLE 1. Producing Agents and Structural Features of Nonpolar Mycosides C

Producing microorganisms	Mycosides	R	k	"X"	Structure of the O-methylrhamnose	Reference
M. avium	C_2	$CH_3(CH_2)_{14}$ and $CH_3(CH_2)_{16}$	1	Alaninol	D	[176]
M. avium 802	C_2	$CH_3(CH_2)_{22}CH=CHCH(OCH_3)CH_2$ and $CH_3(CH_2)_{24}CH(OCH_3)CH_2$	1	"	T	[177]
M. butyricum	C_b	$CH_3(CH_2)_{22,24}CH=CHCH(OCH_3)CH_2$	0	N,O-Dimethyl-L-serine Alaninol	T	[177, 178]
"	C_{b1}	"	0		T	[179]
M. scrofulaceum*	C_s	$CH_3(C_nH_{2n-2})CH(OCH_3)CH_2$ $n=28-31$	0	Alaninol	D	[180]
M. marianum*	C_m		2		D	[181]
M. smegmatis	C_{sm}		0		D and T	[182]
Mycobacterium sp. 1217	C_{1217}	$CH_3(CH_2)_{21}CH(OH)CH_2$ "D"	0	Alaninol and ethanolamine	D and T	[172]
"	C_{1217} fr. A	$CH_3(CH_2)_{24}CH(OH)CH_2$ "D"	0	Alaninol	D	[172]
M. farcinogenes 378	C'_{378}	$CH_3(CH_2)_{22,24}CH(OCH_3)CH_2$	0	Alaninol	M and D	[175]

*These species are probably identical.



(LVIII)

(LVIII a) Z = -4,6-(1-carboxyethylidene) 3-O-Me-β-*D*-Glcp (serotype 8)
(LVIII b) Z = -2,3-O-Me_2-α-*L*-Fucp-(4←1)-2,3-O-Me_2-α-*L*-Fucp (serotype 9)
(LVIII c) Z = -2-O-Me-α-*L*-Fucp-(4←1)-2-O-Me-α-*L*-Fucp (serotype 25)

Formally, the polar mycosides C may be regarded as additionally glycosylated nonpolar mycosides (LVII, k = 0). It is possible that the biosynthesis of the former takes place through the glycosylation of the latter. The oligosaccharide parts of the molecules with the polar mycosides are acetylated to different degrees. The corresponding lipid fractions therefore represent a mixture of components differing by the number of O-acetyl groups; however, after alkaline deacetylation they migrate on TLC as individual substances [174, 186]. This fact has made it possible to develop a method for determining the serotypes of the MAIS complex with the aid of the TLC of the deacetylation products of the mycosides C formed by the organisms under consideration [173, 190]. The method permits the identification of all 31 serotypes of the complex and, with a slight modification, is suitable for determining a number of other atypical mycobacteria (*M. kansasii*, *M. szulgai* complex of *M. gordonae*, *M. terrae*, *M. xenopi*, and *M. gastri*), the surface antigens of which, although they have a glycolipid nature, nevertheless probably differ from the mycosides C [190]. It must be mentioned that in recent years the question of identifying atypical mycobacteria has become extremely acute — it has been found that the diseases caused by these organisms are considerably more widely distributed than was previously thought [191], while the seroagglutination tests usually used in clinical medicine frequently prove to be unreliable [190].

The polar and nonpolar mycosides C form the outer coat of the atypical mycobacteria which is observed under the electron microscope in the form of a transparent zone at the periphery of the cells [174, 182, 185, 186]. All, or almost all, the cellular mycosides C are concentrated in it. Not less than 70% of the total weight of the coat is due to the polar mycosides and the remainder to the nonpolar mycosides (~20%) and other lipids. The structural element of the cells that is under consideration consists of longitudinally arranged bundles of paral-

lel fibers, the diameter of each fiber being about 4 nm [186]. The internal part of the mycoside layer is rigidly linked to the cell wall and creates a thin microcapsule; the outer surface (the macrocapsule) is mobile and is readily removed mechanically — for example, when the culture liquid is vigorously shaken. Strains of the MAIS serocomplex have been isolated which have no outer coat, and they contain no mycosides C [193].

In the electron microscopy of the tissues of animals infected with *M. leprae* [194] and with *M. lepraemurium* [195, 196], likewise, zones transparent for electrons surrounding the mycobacteria have been detected. A hypothesis has been put forward [195, 196], according to which these zones represent a protective capsule which permits the pathogen to survive in the macrophages of the host and to multiply even after the coalescence of the phagosoma. *M. lepraemurium* is antigenically related to the MAIS serocomplex [197]. Furthermore, the presence of mycosides C in the capsular material of the former has been established [196]. Thus, there are grounds for considering that the chemical natures of the electron-transparent sheaths of the bacteria mentioned are, at least, close. In this case, the mycosides C must play an important role in the pathogenesis of the diseases caused by the atypical mycobacteria. From the immunological point of view, *M. leprae* has little in common with MAIS serocomplex. However, the capsule of *M. leprae* likewise consists of glycolipids which, on TLC, migrate close to the polar mycosides C and, like them, contain O-methylated 6-deoxyhexoses [198]. It is possible that these glycolipids differ structurally from the mycosides, but their role in the parasitizing microbial cell is similar. It is obvious that the creation of drugs selectively inhibiting the biosynthesis of mycosides C and related capsular lipids would considerably facilitate the fight against the serious diseases caused by nontubercular mycobacteria (leprosy, various mycobacterioses).

Other biological functions are characteristic of the nonpolar mycosides C. It has been shown that the mycobacteria synthesizing mycosides C_b, C_s, C_{sm}, and C_{1217} (see Table 1) are resistant to mycobacteriophage D_4 [199, 200]. The reception and inactivation of the phage take place in sections of the cell surface containing these glycopeptidolipids [201]. Partially purified mycosides are also capable of inactivating phage D_4, and also D_{29}, but they are inert in relation to phages specific for the mycobacteria that do not produce the above-mentioned mycosides [201].

Glycerol Mycolates

The presence in *M. tuberculosis* of glycerol mycolates and of diglycerides with a mycoloyl residue and a residue of an unsubstituted fatty acid (C_{16}, C_{18}) was reported even in the 50's [202, 203], but the localization of the mycoloyl residue in the molecules was not determined. In recent years, a series of papers devoted to the isolation of glycerol mycolates from organisms related to the mycobacteria has been published. Fractions of 1-O-mycoloylglycerides have been isolated from the cells of *Nocardia asteroides* [204], *N. rhodochrous* [205, 206], *Corynebacterium pseudotuberculosis* [207], *Gordona lentifragmenta* and *G. bronchiales* [208]. With the aid of spectrometric methods it has been established that in all the monoglycerides obtained the mycolic acid residues were bound to a primary HO group of the glycerol. The absolute configurations of the glycerol residues were not determined by the authors of the papers mentioned. It is striking that no monoglycerides of low-molecular-weight fatty acids, which form components of the usual membrane lipids of bacterial cells (glycerophospholipids and neutral glycerides) were found in the lipids of the bacteria mentioned. On the other hand, a culture of *Corynebacterium rubropertincta* contained just such low-molecular-weight monoglycerides with $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, and tuberculostearic acid residues, but the cells of this culture contained no glycerol mycolates [208]. It is assumed that in the biosynthesis of the mycoloylglycerols the transfer of a mycolic acid residue to the glycerol molecule is effected by specific enzyme systems differing from the usual acyl transferases. The localization in the cells and the functions of the monoglycerides under consideration are unknown.

We have mainly discussed those classes of specific lipids of mycobacteria and related organisms that have aroused the greatest interest among research workers in recent years. It is obvious that the synthesis by the bacterial cell of unusual lipids is due to their requirement in the performance of certain special physiological functions. The combined study of these functions and the chemical structure of the lipids corresponding to them is not only necessary for theoretical conclusions in the link between the structural features of natural compounds and their biological roles. It also becomes of practical importance when it is a question of pathogenic microorganisms, since the routes to the biosynthesis of specific lipids may be selected as the target for newly created drugs. The available results of laboratory

investigations of the biological activity of the cord factor and its natural and synthetic analogs permit a real hope that these glycolipids will find use in medicine as agents for the treatment of malignant tumors and immunodeficiency states. It must be emphasized that many classes of specific lipids of mycobacteria have not been studied at all in relation to their biological activity and, probably, by no means are all classes of such lipids known. It may be expected that the further all-sided investigation of the lipid compositions both of mycobacteria and of other microorganisms widely distributed in nature will lead to important theoretical and practical results.

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