A THEORY OF BIOGENESIS OF LICHEN DEPSIDES AND DEPSIDONES

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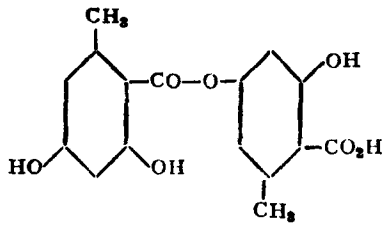
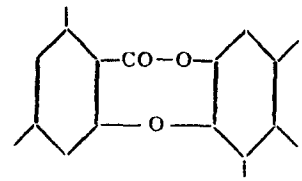
Lichens as a group are interesting not only botanically but also chemically. They are large in number and occur almost all over the globe. They have been used as food and also for the preparation of colouring matters, drugs and perfumes. In several respects their chemistry is unique. As reserve carbohydrates they possess lichenin and isolichenin instead of starch, and they frequently contain considerable amounts of sugar alcohols such as erythritol and mannitol. But their most characteristic components are the lichen acids which seem to be built on an altogether original pattern.

The first attempt at a classification of lichen components was made by Zopf in his book on lichens published in 1910. As the result of the large volume of later work which led to a clearer understanding of their chemistry, Zopf's scheme became obsolete and a better classification was given by Asahina[1] in 1934. He places them into three main classes: (A) compounds of the aliphatic and alicyclic series, (B) compounds of the aromatic series, and (C) substances of unknown nature. In each of the first two there are several groups and subgroups. Depsides form group IV and Depsidones group V under (B). They have the primary skeletons (I) and (II) respectively; the former corresponds to phenyl-benzoate and the latter has an oxygen bridge in addition. Each group has again been divided into two sub-groups, (1) Orcinol derivatives having nucleus (III) and (2) β-orcinol derivatives having nucleus (IV). A large number of these compounds are known and the constitutions of most of them are definitely established. When all the available data are carefully examined certain features come out strikingly and it seems to be possible to develop a general theory of biogenesis which may be useful for further work.

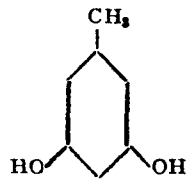
Though depsides and depsidones have been divided by Asahina into four groups for purposes of classification, structurally they are closely related and biogenetically they all seem to belong to one group. In the lichens, depsides and depsidones, orcinol and β-orcinol derivatives all occur together



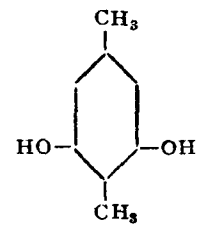
I depside



II depsidone



III Orcinol



IV β-Orcinol

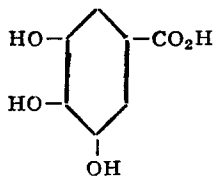
as the select examples given below (Table I) will show. It seems to be therefore definite that they are evolved from the same primary compound, the variations being brought about by processes of oxidation and reduction, and other simple reactions.

TABLE I

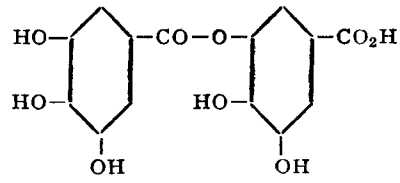
Parmelia abessinica	..	lecanoric acid (*a*), atranorin (*b*), salazinic acid (*d*).
P. Physodes and *furfuracea*	..	atranorin (*b*), physodic acid (*c*).
Evernia prunastri	..	evernic acid (*a*), atranorin (*b*).
Cetraria collata	..	atranorin (*b*), α-collatolic acid (*c*).
Lobaria pulmoria	..	gyrophoric acid (*a*), stictinic acid (*d*).
Usnea species	..	atranorin (*b*), protocetraric acid (*d*).
(*a*) = orcinol depside,		(*b*) = β-orcinol depside,
(*c*) = orcinol depsidone,		(*d*) = β-orcinol depsidone.

Comparison of lichen and tannin derivatives.—In recounting the characteristic features of the lichen acids, it may be useful to compare them with tannin derivatives. The latter are based on gallic acid (V *a*) and consist of meta-digallic acid units (V *b*). Frequently these are in combination with glucose or some other sugar. Lichen depsides (VI *b*) on the other hand are based on orsellinic acid (VI *a*) (including substituted or derived orsellinic acid) units. Occasionally they are found in combination with the sugar alcohol, erythritol as esters. Though they are mostly didepsides, some tridepsides are known such as gyrophoric acid, tenuiorin and umbilicaric acid. The majority of them are para-depsides and it seems to be due to the fact that in orsellinic acid no meta-hydroxyl is present. However if conditions should be favourable and if a meta-hydroxyl is made available by nuclear oxidation, meta-depsides result. Sekikaic acid, ramalinolic acid

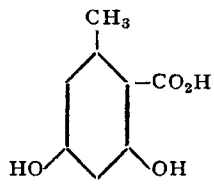
(XXXI), thamnolic and hypothamnolic[2] acids (XXXII) belong to this group of meta-depsides though some of them were earlier considered to be para-depsides. In view of the large occurrence of *m*-depsides in tannins and of Fischer's findings that acyl groups migrate readily from a para to a meta oxygen atom in gallic acid derivatives, meta-depsides should be more stable and may be expected to be found in the lichens also whenever meta-hydroxyls are present.



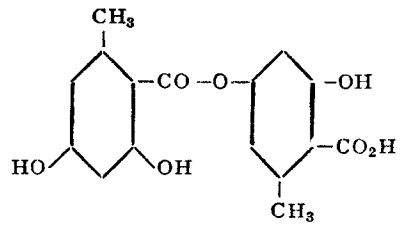
V*a* gallic acid



V*b* meta-digallic acid

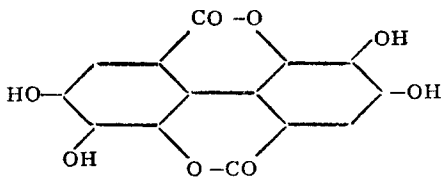


VI*a* Orsellinic acid

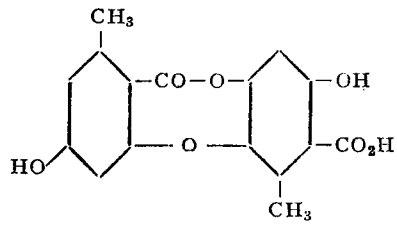


VI*b* Lecanoric acid (*p*-depside)

There is one other item of comparison and contrast between the tannin and lichen components. Ellagic acid (VII) could be compared with the lichen depsidones (VIII). Both seem to arise from nuclear oxidation or dehydrogenation of the corresponding depsides. The former is a diphenyl derivative whereas the latter has a diphenyl ether group. This difference is again due to structural restrictions since in orsellinic acid no free ortho-position is available and an ortho-hydroxyl has to be used for linking the two phenyl nucleii.



VII



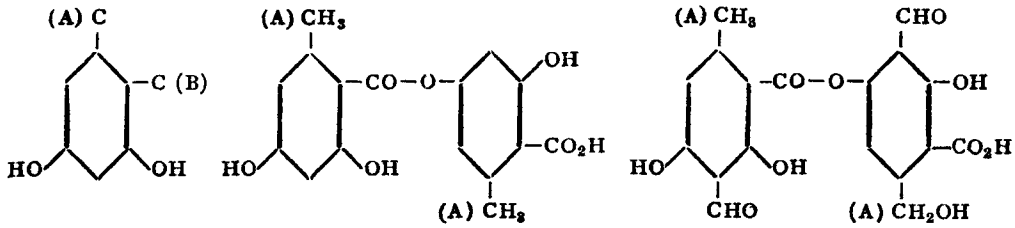
VIII

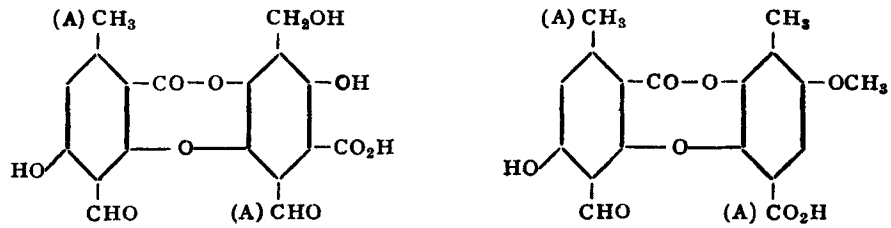
Occurrence of orsellinic acid unit (C_8 *unit*).—Though gallic acid which is the unit of tannin depsides occurs free in nature there was for a long time no definite proof of the occurrence of free orsellinic acid or its simple derivatives.

When esters of these acids were isolated in certain cases their presence was attributed to alcoholysis[3] taking place during the course of extraction. The discovery of montagnetol (*d*- as well as *dl*-) in the Indian lichen, *Roccella montagnei* by Rao and Seshadri[4] may therefore be said to be the first definite case of the existence of the single orsellinic acid unit. It is the erythrityl ester of orsellinic acid and has been obtained by methods of extraction which cannot involve any change in the nature of the components. Till recently there was the possibility that erythrin also might belong to this category; it interested us on this account. In Zerner's formula for this substance two orsellinic acid units are independently linked with one molecule of erythritol. The question of its constitution was raised in one of our publications in 1940[4a] and we announced that work was already in progress. It has now been independently investigated by Sakurai[5] and by Rao and Seshadri[6] who have shown definitely that it is the erythrityl ester of lecanoric acid. The work of the latter authors on this subject was complete in June 1941 and the paper was communicated for publication in April 1942. Due to the difficult conditions of war, particularly about that time, information about Sakurai's work reached us through the medium of the *British Chemical Abstracts* only after our paper had been published. Though the two investigations were planned somewhat differently the results common to both agree closely.

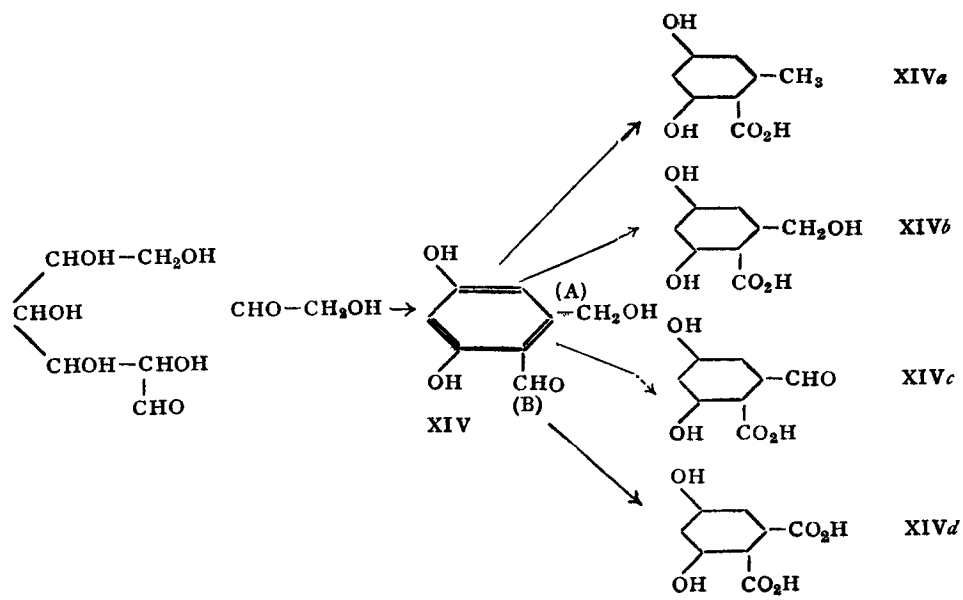
Free orsellinic acid has not so far been found in lichens ; orcinol is however present along with lecanoric acid in *Roccella tinctoria.* It definitely accompanies montagnetol in *Roccella montagnei.* The explanation of these observations already offered by us[7] seems to be satisfactory. Orsellinic acid is too unstable to remain free. It has to be stabilised by depside formation or by esterification with erythritol; otherwise it undergoes decarboxylation to yield orcinol.

The foregoing discussion and a careful review of the structures of all known lichen depsides and depsidones lead to the conclusion that the C_8 skeleton (IX) characteristic of orsellinic acid is the basis for all of them. This is unique for lichens and does not seem to occur elsewhere. Orsellinic acid itself cannot be the fundamental substance, because though carbon atom (B) in the C_8 unit is always present as a carboxyl, carbon atom (A) is in different states of oxidation in a large number of the lichen acids and can be either CH_3, CH_2OH, CHO or CO_2H. Typical examples taken from different groups of lichen compounds are given below. In the structures of the molecules though there are other features, the orsellinic acid units alone should be considered; carbon atom (A) is marked.





Origin of the C_8 unit.—It is now suggested that as the first stage in phytochemical synthesis a reactive substance of the C_8 type (XIV) is produced from a molecule of hexose and one of biose by condensation of the aldol type and elimination of water, and modifications arise subsequently due to oxidation and reduction leading to the existing variations. Out of the four modifications, the first one (XIV *a*) is the most important and it seems to involve internal oxidation and reduction (internal oxygen adjustment). The possibility of XIV *b* or *c* giving rise to the others also exists.

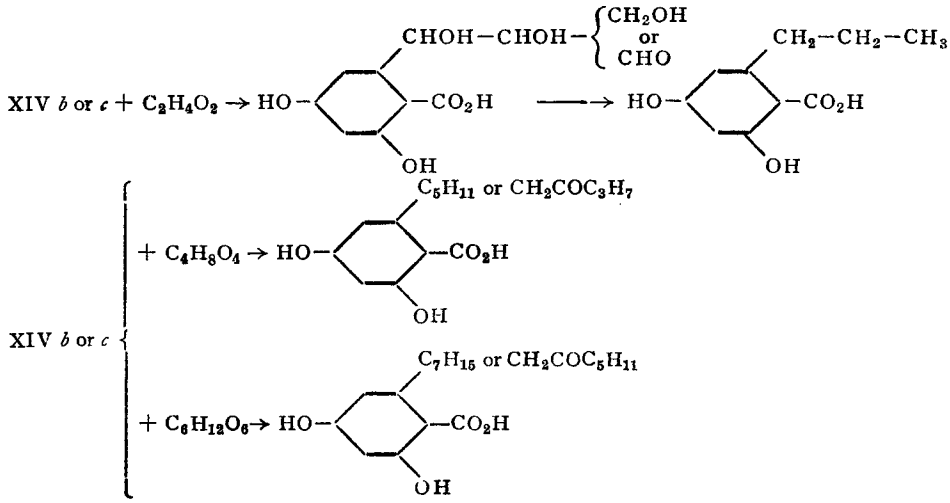


Evolution of longer side-chains.—In several lichen acids longer chains having odd number carbon atoms (3, 5 and 7) occur in the same place as (A). The interesting points to be noted in this connection are, (1) different chain lengths are found frequently in the same depside molecule and (2) in lichen thalli, compounds with one carbon atom in position (A) occur side by side with others having 3, 5 or 7-carbon-atom chains instead. It seems to be therefore reasonable to consider that the longer chains are the result of subsequent building up from the C_8 skeleton. In support of this idea it may be mentioned that frequently the second carbon atom in the chain and exceptionally the first are involved in a carbonyl group and this feature may be taken as an indication of the junction of two parts. Typical cases are given below:

(*a*) Lichen acids having different side-chains in the two halves: sphærophorin (CH_3 and C_7H_{15}), ramalinolic acid (C_3H_7 and C_5H_{11}), olivetoric and physodic acids (CH_2-CO-C_5H_{11} and C_5H_{11}), glomelliferic acid (CH_2-CO-C_3H_7 and C_5H_{11}), lobaric acid (CO-C_4H_9 and C_5H_{11}).

(*b*) Association of lichen acids with different chain lengths in the same lichen: Obtusatic acid (C_1) and sekikaic acid (C_3) occur together in Manchurian Ramalina. Atranorin (C_1) accompanies olivetoric acid (C_7 and C_5) in *Evernia furfuracea*, α-collatolic acid (C_7) in *Cetraria collata* and *Lecanora altra*, and divaricatic acid (C_3) in *Anzia japonica*. Salazinic acid (C_1) occurs together with lobaric acid (C_5) in Lobaria.

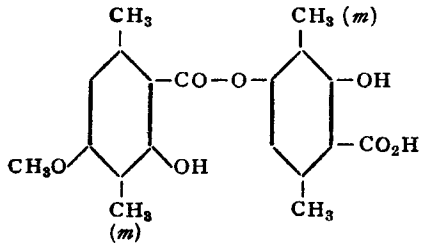
From the above data it may be concluded that derivatives of divarin (C_3), olivetol (C_5) and sphæropherol (C_7) are obtained from (XIV) or more probably from (XIV*b*) or (XIV*c*) by aldol condensation with biose, tetrose



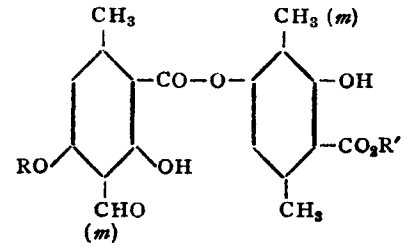
and hexose respectively and subsequent reduction. There is no direct support for the occurrence of biose in the lichens, but the availability of tetrose and hexose is indicated by the occurrence of erythritol, and of mannitol, lichenin and isolichenin. In the following discussion the term 'C_8 unit' includes these derivatives with longer chain lengths also.

Depsides: (i) *Orcinol derivatives.*—Depsides which are orcinol derivatives can be obtained by the combination of any two of the units XIV (*a*), (*b*), (*c*) and (*d*). Variations can again arise in the length of the side-chain methylation of the hydroxyl groups and esterification of the carboxyls. A very large number of possibilities therefore exist though many of them have not yet been found in nature. Whether all the above modifications take place prior to depside formation or after it, is not possible to say and may not be quite necessary for the present discussion.

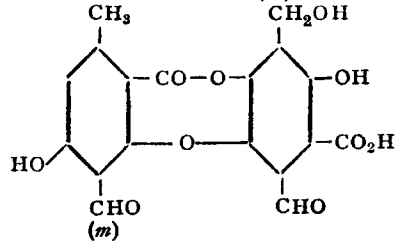
β-Orcinol derivatives: *nuclear methylation.*—Depsides which are β-orcinol derivatives are characterised by the presence of a susbtituent in the nuclear position between the two phenolic hydroxyl groups of the orsellinic acid unit. The substituent is not always a methyl group; it is frequently a carbinol, an aldehyde and even a carboxyl group. The following examples taken from both depsides and depsidones illustrate the point. The concerned nuclear position which is meta to the carboxyl is marked (*m*).



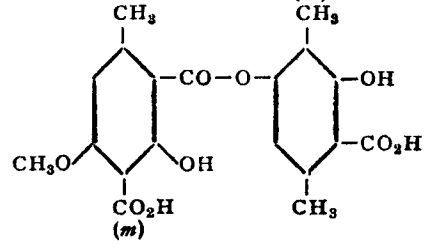
XV Barbatinic acid



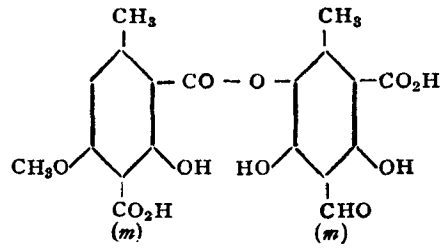
XVI Atranorin : R=H, R'=CH_3.
Beomycessic acid : R=CH_3, R'=H



XVII Salazinic acid

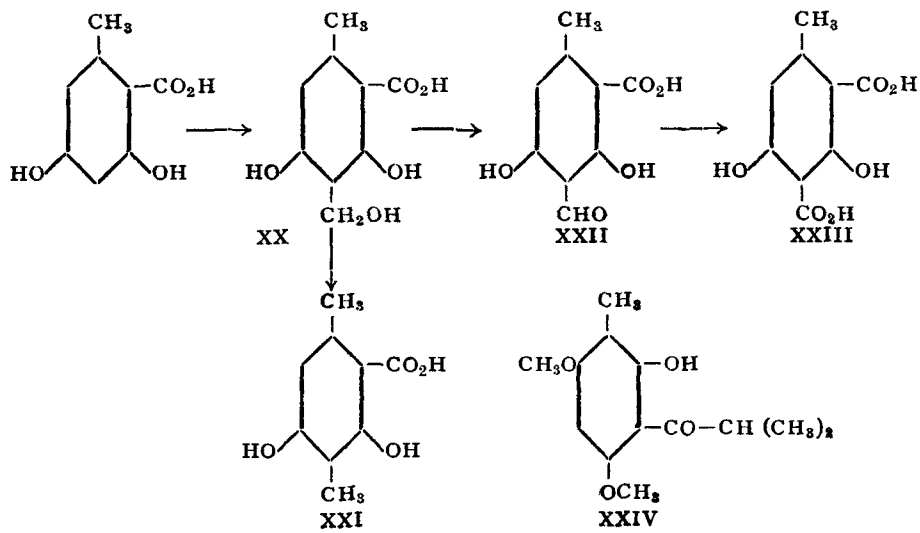


XVIII Squamatic acid



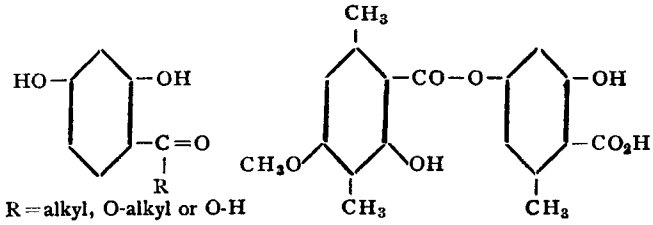
XIX thamnolic acid

The position involved in the substitution is exactly where nuclear methylation in orsellinic acid and esters takes place in the laboratory and hence the formation of β-orcinol derivatives in lichens may be attributed to a similar process. The role of formaldehyde as a methylating agent for hydroxyl and amino groups is well known. The reaction involves the removal of oxygen as an essential stage. This may be effected by intramolecular adjustment as in the biogenesis of alkaloids[8] and also by means of some other reducing substances present in the system. In the laboratory the reagent itself serves this purpose and part of it undergoes oxidation. The reasonable assumption is made here that nuclear methylation is also brought about in the plant by means of formaldehyde. This explains very satisfactorily the existence of various stages of oxidation in the new side-chain. The primary product of the condensation of formaldehyde will be the carbinol (XX). If it suffers reduction a methyl group will be produced (XXI) as is found in most cases in lichen acids. But the carbinol can be left as it is or it can be oxidised to the aldehyde (XXII) and carboxyl stage (XXIII). The occurrence of lecanoric acid, atranorin, and salazinic acid together in



Parmelia abessinica and of beomycessic acid (XVI) and squamatic acid (XVIII) in *Thamnolia vermicularia* provide data in support of the above contention. A close laboratory analogy to this type of reaction is nuclear formylation using hexamine leading to the formation of aromatic hydroxy aldehydes.[9] The term 'nuclear methylation' is employed in the sequel in a general sense for the entry of the new side-chain and includes all the possible stages of oxidation given above. Nuclear methylation so facile in lichen acids, is not common elsewhere in natural products, but appears to take place occasionally when the conditions are favourable. Bæckeol[10] (XXIV) may be considered to be an example of a compound resulting from facile nuclear methylation.

The mechanism of nuclear methylation of phenolic-carbonyl compounds of the type (XXV) has been discussed in one of the past publications[11] from this laboratory. Crabtree and Robinson[12] have shown that a free hydroxyl in the 4th position is necessary for the reaction. It may therefore be concluded that in lichen acids nuclear methylation should precede depside formation, at least as far as the right half is concerned. Almost all β-orcinol derivatives have nuclear methylation in both the C_8 units involved. There is however a case of mixed depside in obtusatic acid (XXVI) and this lacks nuclear methylation in the right half. It is probably an instance where the reaction did not take place before depside formation and the left half got methylated after the depside stage. The occurrence of obtusatic acid along with evernic acid which is free from nuclear methylation in the two halves, in certain Ramalina species may be significant in this connection. But in general nuclear methylation may be said to precede depside formation. An important feature, which is in accordance with the above idea, is that in the lichens nuclear methylation is not necessarily accompanied by methylation of the neighbouring *p*-hydroxyl group and does not therefore interfere with subsequent depside formation.

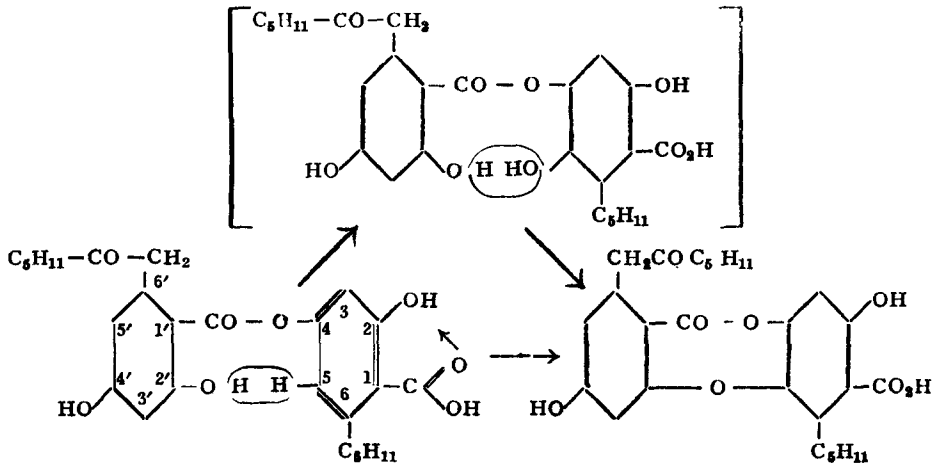


XXV XXVI obtusatic acid.

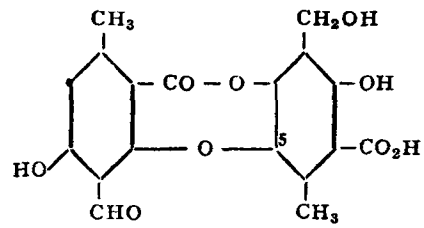
At this stage the large scope for the existence of different β-orcinol depsides may be considered. The β-orcinol halves can have variations in the original side-chain (A) of the C_3 unit and also in that arising from nuclear

methylation. Several combinations of these units can be chosen for depside formation. Further, methylation of the hydroxyl groups and esterification of carboxyls add to the possibilities.

Depsidones.—It has already been explained that the formation of depsidones involves oxidation or dehydrogenation leading to the establishment of an oxide link between two C_8 units. Depsidones seem to be based on depsides and represent a later stage in evolution. The relation between physodic acid (XXVIII) and the corresponding depside, olivetoric acid (XXVII) may be represented as below:



XXVII Olivetoric acid XXVIII Physodic acid



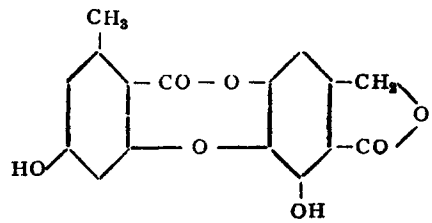
XXIX Protocetraric acid

The depside microphyllenic acid corresponds similarly to the depsidone α-collatolic acid.

In support of the above conclusion the following characteristics may be mentioned. In the left half of the molecule the ortho-hydroxyl (2'-position) has always to be used, but in the right half there are two alternative nuclear positions, 3 and 5, available for this purpose. Depsidone formation, however, seems to involve invariably position 5. This may be inevitable in β-orcinol derivatives (*e.g.*, proto-cetraric acid XXIX) since

nuclear methylation taking place earlier should have already used up position 3. The use of the 5th position even in orcinol derivatives indicates definitely that the reactions leading to depsidone link take place only after the depside stage, because the conditions for the preferential activation of the 3-position will not now exist. The hydroxyl in position 2 is now the activating group and the 5th position which is para to it, is activated more easily for purposes of oxidation or dehydrogenation. The possible fixation or preferential orientation of aromatic double bonds due to the existence of chelation[13] may also contribute to the lack of activity of position 3 and the comparatively greater reactivity of position 5 (see formula XXVII).

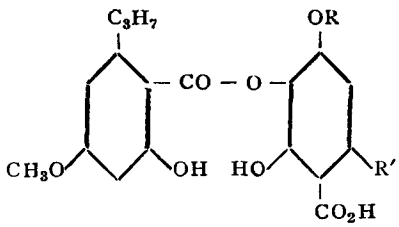
Quite recently Murphy, Keane and Nolan[14] have attributed to variolaric acid isolated from *Lecanora parella* the formula XXX. It is an orcinol derivative and the structure suggests that the 3-position is involved in the depsidone link. It seems to be therefore exceptional. However, it may be necessary to wait for a confirmation of this constitution before a variation in the above scheme of depsidone formation is recognised.



XXX Variolaric acid

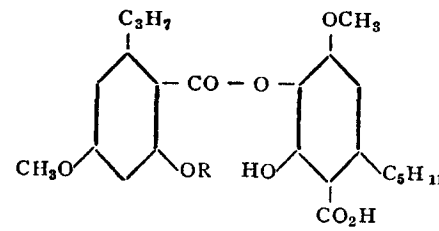
Meta-depsides.—Nuclear oxidation without leading to depsidone formation is also found in lichen acids. In most of these cases meta-depsides result. Sekikaic and Ramalinolic acids (XXXI) are two orcinol depsides in which the 3-position has undergone oxidation. They are closely related and are found to occur together in Ramalina species. Homosekikaic and boninic acids (XXXI *a*) are a similarly related pair. On the other hand, thamnolic and hypothamnolic acids (XXXII) are related β-orcinol depsides in which oxidation of position 5 is involved. With regard to their biogenesis it is significant that these *m*-depsides are associated with *p*-depsides in lichen thalli. Obtusatic (XXVI) and sekikaic (XXXI) acids occur together in Manchurian Ramalina; squamatic (XVIII) and thamnolic (XXXII) acids are found together in *Thamnolia varmicularis*. Thus there is indication that the two types have similar origin. It seems to be correct to consider that nuclear oxidation of the 3- or the 5-position takes place in the C_8 unit concerned prior to depside formation and since a meta-hydroxyl group is thus rendered available, a meta-depside results. The reactive position

will be the 3- in orcinol units and the 5- in β-orcinol units. The other alternative (*i.e.*) oxidation at the ordinary *p*-depside stage does not seem to be satisfactory and is not applicable to all cases as explained below. In the *p*-depsides the 3-position becomes unreactive and hence the formation of sekikaic, ramalinolic and related acids (XXXI) cannot be explained. Though the oxidation of the 5th position can take place after the depside stage, isomeric change to meta-depsides should then be assumed to occur subsequently under the conditions of the plant, and at the same time normal depsidone formation should be inhibited. The view that all the meta-depsides are due to oxidation taking place prior to depside formation is free from these defects and may therefore be accepted until new data render a change necessary.



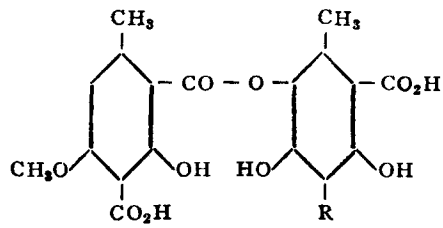
R=CH_3, R′=C_3H_7, sekikaic acid
R=H, R′=C_5H_{11}, ramalinolic acid

XXXI



R=H, homosekikaic acid
R=CH_3 boninic acid

XXXI*a*



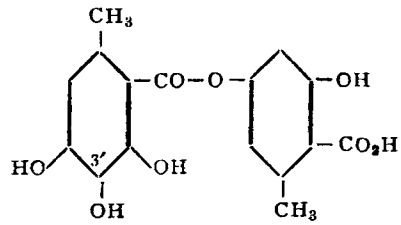
R=CHO, thamnolic acid
R=CH_3, hypothamnolic acid

XXXII

Though the above depsides could be expected to undergo dehydration and form depsidones, the corresponding depsidones do not seem to have been recorded. It is possible that they will be discovered in future, or there may be special reasons for the stability of these meta-depside molecules. Future work may throw light on this subject.

In the examples given above (depsidones as well as *m*-depsides) nuclear oxidation involves only the right half of the molecules concerned. There is one case where the left half is affected. It is diploschistesic acid[15] isolated from *Diploschistes scruposus* and having the constitution XXXIII. In

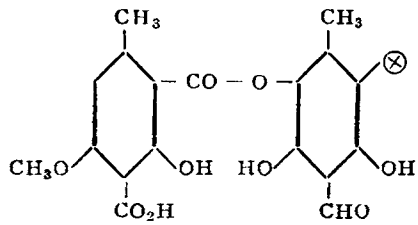
it nuclear position 3′ which is the most reactive in the left half, has undergone oxidation, possibly even after depside formation.



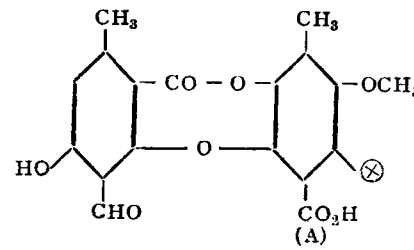
XXXIII Diploschistesic acid

In lichen acids the occurrence of methylation of the hydroxyl groups situated ortho to carboxyl groups is not unusual. Even in the laboratory these groups of the lichen depsides and depsidones undergo methylation under comparatively mild conditions using diazomethane or methyl iodide and potassium carbonate. This facility of methylation may be attributed to the weakening of the chelation existing between the concerned carbonyl and hydroxyl groups, the weakening being caused by the existence of substituents in positions ortho to these groups.[16]

To some extent decarboxylation of the lichen acids seems to take place in the plant. Decarboxylation is known to occur in the course of preparation on a large scale, particularly when the sample is boiled for a long time with solvents. The occurrence of decarboxy-thamnolic acid[2] (XXXIV) in commercial samples of thamnolic acid is attributed to this cause. The probable formation of orcinol from orsellinic acid has already been mentioned. A similar example belonging to the depsidone group seems to be psoromic acid[17] (XXXV) in which the original carboxyl (B) of the right half is lost; the one that remains seems to represent (A). The positions of the lost carboxyls are marked in the formulæ given below:



XXXIV Decarboxy thamnolic acid



XXXV Psoromic acid

Summary

Lichen depsides and depsidones are considered to arise from a common source (XIV) which originates from aldol condensation between a hexose and a biose and elimination of water. Oxidation and reduction lead to

various modifications of this C_8 unit and increase in the length of the side-chain arises from condensation with simple sugars and reduction. Depsides are formed by the combination of two of these units. β-Orcinol derivatives are obtained by nuclear methylation by means of formaldehyde and this reaction in general takes place prior to depside formation though the other possibility is not altogether excluded as far as the left half is concerned Depsidones come last in the evolution; they are based on depsides and require oxidation or dehydrogenation involving position 5 which is para to the activating hydroxyl. Nuclear oxidation without leading to depsidone formation also occurs. Either the 3- or the 5-position is involved and meta depsides result. Oxidation involving the left half is also possible and is represented by diploschistesic acid. The occurrence of orcinol and psoromic acid is attributed to decarboxylation taking place in the plant.

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Note.*—Formaldehyde has been shown by Hanus (*B. C. Abs.* AII, 1944, 14) to be capable of introducing carbinol (CH_2OH) groups into the nucleus of ortho-cresol in an alkaline medium. It has also been used for building this group on a ternary carbon atom in the course of the synthesis of Pantothenic acid (Stiller *et al.*, *J. A. C. S.*, 1940, 1785) and such a reaction has been considered to take place in the biogenesis of the above acid by Kuhn and Wieland (*B. C. Abs.* A.II, 1942, 249). The method of nuclear methylation of phenolic substances using formaldehyde in the presence of a mineral acid described by Burawors (*Nature*, 1943, **151, 615) is rather complex in its mechanism.