Naphthoquinone Biosynthesis in Molds. The Mechanism for Formation of Mollisin*

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ABSTRACT: The biosynthesis of mollisin, 8-dichloroacetyl-2,7-dimethyl-5-hydroxy-1,4-naphthoquinone, was studied by addition of radioactive substrates to solid agar cultures of *Mollisia caesia*. Labeled acetate and malonate were good precursors of mollisin. Methyl-labeled methionine, [2-^{14}C]mevalonic acid (or lactone), and labeled chloroacetic and bromoacetic acids were not utilized for mollisin biosynthesis. Degradation of mollisin samples from the acetate and malonate experiments indicated a fundamental role for the acetate plus polymalonate pathway in mollisin biosynthesis. The addition of bromide ion to growth media did not result in the diversion of the biosynthetic pathway to a bromo analog of mollisin. A red oil, isolated during the purification of crude mollisin samples, was shown to contain 2,7-dimethylnaphthazarin.

Little is known about the biosynthesis of naphthoquinone pigments formed by various molds. In a study of the mechanism for formation of javanicin, compound I (see Scheme 1), we have observed that this naphthoquinone is synthesized by the "acetate plus polymalonate" pathway (Gatenbeck and Bentley, 1965). In particular, the methyl group attached directly to the naphthoquinone nucleus is derived from the carboxyl of acetate or malonate rather than from a C_1 donor such as methionine. A yellow pigment, named mollisin, had been observed by Gremmen (1956) to be produced when strains of the *Discomycetes*, *Mollisia caesia* and *Mollista fallens*, were grown on malt agar media. At the start of our work on javanicin, Kerk and Overeem (1957) had concluded that mollisin was a unique, chlorine-containing naphthoquinone, with the structure II. Since structure II could be derived from the same theoretical polyketomethylene intermediate, compound IV, as javanicin, although by cyclization at different positions, we also undertook a study of mollisin biosynthesis. When this work was under way, we were informed by Dr. Overeem[1] that it was necessary to revise the structure of mollisin to compound III (see Overeem and Kerk, 1964a,b). Although no longer related to javanicin, this structure possessed intrinsic biosynthetic interest, particularly with reference to the origin of the two methyl groups linked to the naphthoquinone nucleus.

Experimental

Production of Mollisin. The organism used in this work was *Mollisia caesia* Sacc. sensu Sydow, strain number 170 of Gremmen, obtained from Centraalbureau voor Schimmelcultures, Baarn, the Netherlands. It was maintained on slopes of malt agar (Oxoid), the incubation temperature being 21–23°; temperatures of 28–29° prevented growth of the organism. For inoculations, a piece of mycelium and agar, about 2 mm square, was transferred to the new slope. A total of approximately 1.0 g of mollisin was obtained from several batches of *M. caesia* cultures, each batch consisting of thirty Petri dishes (diameter, 13.5 cm). In these experiments, and some of those in which labeled substrates were added, the medium had the following composition: malt extract agar (Oxoid), 50.0 g; NaCl, 0.5 g; water, 1000 ml. The addition of NaCl had been observed by Overeem to increase the yield of mollisin.[1]

After 3 weeks, the crystalline pigment formed on the surface and in the body of the agar was extracted with chloroform as described by Kerk and Overeem (1957). Rather than carrying out a number of recrystallizations of the material obtained by evaporation of the chloroform, the extract was sublimed using a cold-finger sublimation apparatus of large capacity. At oil pump pressure (about 1 mm), a first sublimate formed at bath temperature between 85 and 100°; this material, a characteristic red oil, is discussed under Results. Mollisin itself sublimed as the major crop of bright-yellow solid, at bath temperatures between 110 and 145°. After recrystallization as described by Kerk and Overeem (1957), or in better yield from glacial acetic acid, mollisin had mp 203–204°. This material gave all of the color reactions described in the literature; in addition, with magnesium acetate in methanol, mollisin gave a fine purple color.

Tracer Experiments. For experiments with labeled substrates, cultures were best grown on 10 ml of the

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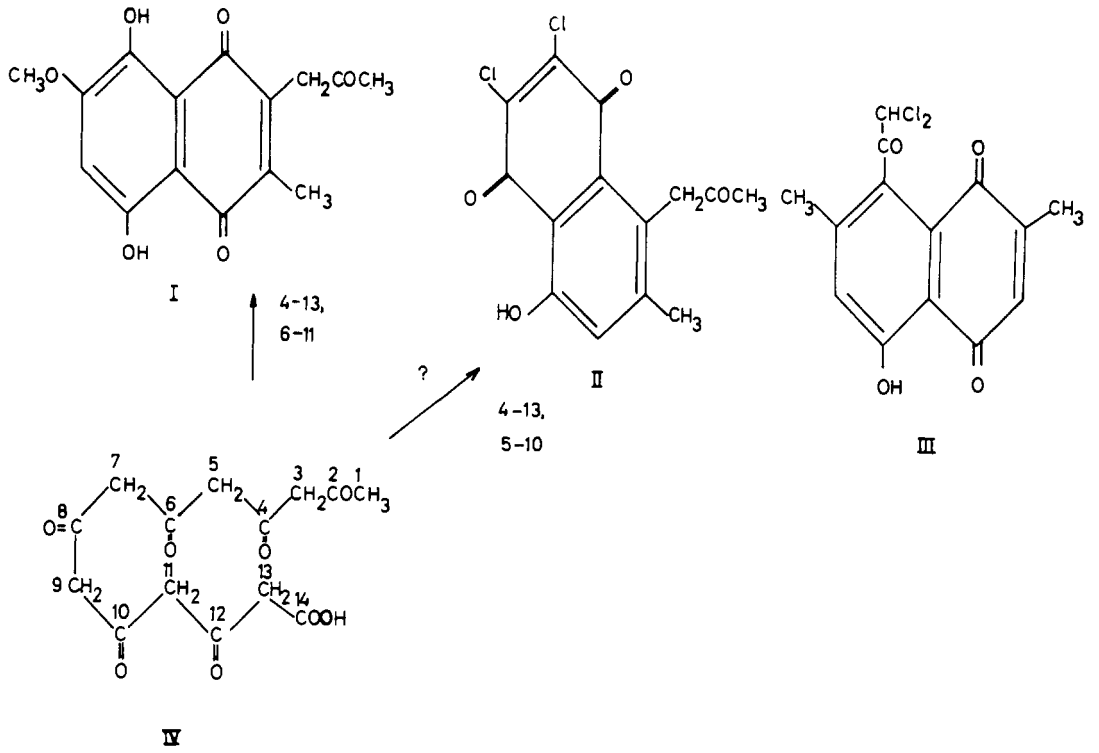
[1] J. C. Overeem, personal communication.



SCHEME 1

malt agar–NaCl medium in tubes 150 × 20 mm. Additions of substrates were made around the base of the mold colonies, with a micropipet. The pipet was sterilized by standing in ethanol, followed by careful flaming prior to use. At harvest, 20 ml of chloroform was added to the culture and the tube was allowed to stand overnight. The yellow solution was removed and replaced with a further 15 ml of chloroform; after again standing overnight, the solution was combined with the first chloroform extract. The combined extracts were extracted twice with 6-ml portions of saturated $NaHCO_3$ solution, then twice with 8-ml portions of water. The chloroform solution was filtered and evaporated to a volume of 20 ml. The amount of mollisin in these extracts was determined by measurement of the absorbance of the solution at 420 mμ, using the value $E^{1\%}_{1cm} = 105.7$ (Kerk and Overeem, 1957).

For paper chromatography of mollisin on a small scale, Whatman No. 1 paper, first soaked with 25% dimethylformamide in alcohol, then air dried, was used with ligroin saturated with dimethylformamide as the solvent (Tarbell *et al.*, 1955); in this system, mollisin had $R_F = 0.10$. Larger mollisin samples from isotope experiments (up to 1.5 mg) were chromatographed on sheets of Whatman 3MM paper (6.5 × 30 cm) previously soaked in 25% dimethylformamide in ethanol; in these cases, less tailing was obtained if the solvent was ligroin, 40; chloroform, 20; and dimethylformamide, 5. Mollisin could also be chromatographed on Vaseline-impregnated paper using propanol-water, 4:1, as solvent (Olson *et al.*, 1961); the R_F was 0.90. However, it was found that this system did not separate mollisin from a radioactive impurity present in experiments when [$^{14}CH_3$]methionine was used as the substrate. This impurity, however, was separated in the ligroin-dimethylformamide system. To remove the mollisin from the papers, the pigment area was cut into small pieces; these were then boiled briefly with changes of glacial acetic acid, until no further yellow pigment was removed. The glacial acetic acid was then removed *in vacuo*. In the study of mollisin derived from labeled mevalonate, the material was eluted from the strips by washing slowly and continuously with methanol containing 20% (v/v) dimethylformamide.

After mollisin had been removed from the culture in a tracer experiment, the agar was melted in a hot-water bath and then diluted with boiling water. The mold colonies were filtered off and were washed repeatedly with boiling water. Following drying in the oven and weighing, the brittle mass was powdered for radioactivity determination as subsequently described.

Radioactivity determinations on mollisin and other compounds were made by total combustion according to Van Slyke and Folch (1940); the carbon dioxide evolved was precipitated as $BaCO_3$. Accurately weighed samples of $BaCO_3$ in the range of 15–20 mg were then suspended in 10 ml of 0.5% diphenyloxazole in toluene with the aid of 400 mg of Aerosil gel for radioactivity determination in the liquid scintillation spectrometer. Kuhn-Roth oxidations were carried out using the oxidizing mixture of Eisenbraun *et al.* (1954); the evolved CO_2 was precipitated as $BaCO_3$ while the acetic acid was re-

TABLE I: Utilization of Radioactive Precursors by *Mollisia caesia*.

Medium	^{14}C Substrate	Additions (days)	Harvest (Day)	Mollisin Yield (mg)	Mollisin Incorporation of ^{14}C (%)	Mycelium Yield (mg)	Mycelium Incorporation of ^{14}C (%)
Malt agar[a]	[$^{14}CH_3$]Methionine	4, 5, 6, 8[b]	12	0.41	0.35[c]	19.6	7.7
Malt agar[a]	[1-^{14}C]Acetate	4, 5, 6, 8[b]	12	0.54	1.57	35.4	26.9
Malt agar, NaCl[d]	[$^{14}CH_3$]Methionine	6, 7, 9, 11[b]	19	2.52	2.66	55.1	23.1
Malt agar, NaCl[d]	[2-^{14}C]Acetate	5, 8[e]	13	1.53	3.80	37.6	9.9
Malt agar, NaCl[d]	[1,3-^{14}C]Malonate	6, 7, 9, 11[b]	19	2.40	1.98	53.8	7.8
Malt agar, NaCl[d]	[2-^{14}C]Malonate	6, 7, 9, 11[b]	19	2.14	6.25	56.4	22.1

[a] These experiments were carried out on 5 ml of medium in a test tube, 150 × 15 mm. [b] Amounts (25 μc) of the indicated substrate were added to cultures on each of the days listed in this column. [c] The incorporations recorded are those determined on the chloroform extract following washing with sodium bicarbonate; these values probably give a fair approximation of the actual incorporations except in the methionine experiments where subsequent chromatography indicated that most of the radioactivity was not associated with mollisin. [d] These experiments were carried out on 10 ml of medium in a tube, 150 × 20 mm. [e] Amounts (50 μc) of [2-^{14}C]acetate were added on these days.

covered by steam distillation. Schmidt degradations on the acetic acid samples were carried out as described by Phares (1951).

Results

Utilization of C_1, C_2, *and* C_3 *Precursors.* As shown in Table I, [$^{14}CH_3$]methionine, [1-^{14}C]acetate, [2-^{14}C]-acetate, [1,3-^{14}C]malonate, and [2-^{14}C]malonate gave substantial radioactivity in the initial chloroform extract from the cultures after acidic materials had been removed with sodium bicarbonate. On paper chromatography, using Vaseline-impregnated paper with the propanol-water solvent, a major peak of radioactivity was subsequently observed to be associated with the yellow pigment area in all cases. With the ligroin-dimethylformamide system, mollisin obtained in the methionine experiments was found not to be radioactive.[2] Under the same conditions, the mollisin derived from the acetate or malonate experiments still showed a radioactive peak associated with the pigment area. Hence it may be concluded that, although acetate and malonate give rise to labeled mollisin, methionine is not incorporated into the methyl groups of the molecule. That utilization of methionine by the mold had actually taken place is shown by the high incorporation of activity into the mycelial components with this precursor.

Labeling Pattern from Acetate and Malonate. To determine the isotope distribution, labeled samples of mollisin were subjected to Kuhn-Roth oxidation; the acetic acid obtained from this oxidation was further degraded by the Schmidt reaction (see Scheme 2).

The results of these determinations, shown in Table II, indicated that activity from [1-^{14}C]acetate was located at carbons 2 and/or 7. The results are consistent with the presence of six labeled atoms in the mollisin samples derived from [1-^{14}C]acetate and [1,3-^{14}C]-malonate. Although the activity of carbons 2 and 7 could not be determined separately, there seems little reason to doubt that there was equal labeling in each carbon since the values for the Kuhn-Roth carbon dioxide were compatible with this assumption. It is apparent from the values in Table II that no specific incorporation of malonate into a part of the molecule had occurred under the present experimental conditions.

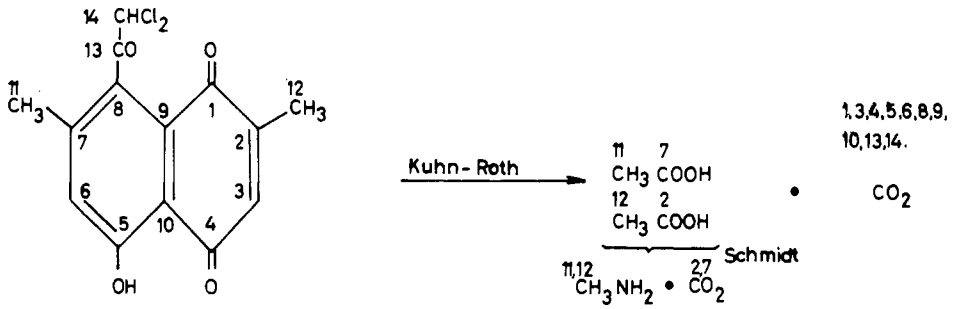
Attempts to Influence the Halogenation Step. The requirement for malt extract agar as a solid growth medium was rather specific. As observed by Gremmen (1956) and Kerk and Overeem (1957), growth on liquid medium was slow and irregular and only small amounts of mollisin were produced in this way; in our experiments, the addition of corn steep liquor or yeast extract did not prove any more successful in liquid cultures than the addition of malt extract to a Czapak-Dox basal medium. On solid malt extract agar our experiments confirmed that addition of NaCl promoted mollisin

[2] A major radioactive peak preceded the pigment area on these chromatograms, and a smaller peak remained at the origin. The possibility that the latter is *O*-methyl mollisin is suggested by the identical behavior of authentic samples.

TABLE II: Chemical Degradations of Labeled Mollisin Samples.

	Mollisin from [1-^{14}C]Acetate[a]			Mollisin from [1,3-^{14}C]Malonate[b]		
	(dpm/mmole mollisin $\times 10^{-5}$)	(number of labeled atoms)	(dpm/mmole labeled atom $\times 10^{-5}$)	(dpm/mmole mollisin $\times 10^{-5}$)	(number of labeled atoms)	(dpm/mmole labeled atom $\times 10^{-5}$)
Mollisin	9.24	6	1.54	5.54	6	0.92
CO_2 from Kuhn-Roth	4.82	4	1.20	3.88	4	0.97
CH_3COOH from Kuhn-Roth: Total	3.12	2	1.56	1.82	2	0.91
COOH	2.80	2	1.40	1.84	2	0.92
CH_3	0.30	0		0.08	0	

[a] In the case of mollisin from [1-^{14}C]acetate, the degradation was carried out on 1.23 mg of purified mollisin diluted with 38.0 mg of carrier orcinol hydrate, since at this time insufficient carrier mollisin was available. The low value in the Kuhn-Roth carbon dioxide could be owing to the fact that some mollisin may have remained unoxidized while the orcinol was completely oxidized. For example, in the later experiment with malonate-derived material, it was observed that even after 4 hours undissolved crystals of mollisin were still present in the flask. This possibility, however, would not affect the determination of the distribution of radioactivity in the acetate. [b] In the case of mollisin from [1,3-^{14}C]malonate, dilution with mollisin itself was carried out according to the following schedule. Purified mollisin (0.94 mg) was diluted with 244.9 mg of carrier mollisin and crystallized from glacial acetic acid. Crystalline mollisin (178 mg) obtained in this way was diluted with a further 158 mg, and the whole was sublimed. A portion of the sublimate (51.2 mg) was used for Kuhn-Roth oxidation.



SCHEME 2

formation. No mollisin was formed on solid Czapek-Dox medium itself, despite the chlorine content of the latter; the agar became dark brown, almost black when this medium was used. Attempts to obtain a bromo analog of mollisin by the addition of bromide to malt extract agar also led to the formation of complex, brown to black pigments in the agar; no yellow pigment was formed on the agar surface in these experiments, so that even the normal mollisin production was repressed. In preliminary experiments aimed at a study of the chlorination step, we found that monochloroacetic acid and monobromoacetic acid labeled with ^{14}C in the carboxyl groups were utilized only very poorly, at most, for mollisin biosynthesis.

Minor Components of Mollisin Fermentations. Purification of the crude mollisin samples by sublimation led to a characteristic, highly volatile, red oil as the first fraction, prior to sublimation of mollisin itself. On standing overnight, the oily material became partly crystalline. Insufficient material was available for complete purification but certain conclusions about this material were possible. The oil gave a characteristic violet-purple color reaction with magnesium acetate in methanol, a gray-green color with alcoholic ferric chloride, and a violet color with zirconium nitrate in acid solution (all reactions carried out on filter paper). These reactions were somewhat reminiscent of javanicin; however, in solvent D of Reio (1958), javanicin had R_F = 0.95, and the red oil had R_F = 0.90–0.92. In cyclohexane solution, the material showed a characteristic spectrum with absorption maxima at 480, 510, 535, and 550 mμ; in addition, a shoulder at 495 mμ was also observed.

The bright-red color of the oil immediately suggested the possibility of a naphthazarin component; in view of the structure of mollisin itself, 2,7-dimethylnaphthazarin seemed a likely possibility. This material was first obtained, along with the 2,6 isomer, by con-

densation of citraconic anhydride with toluhydroquinone in experiments briefly reported by Kuroda (1939). On repeating this condensation, we obtained as the major product a compound with a mp of 125–126° which apparently corresponded with the low-melting form (mp of 127°) of Kuroda. This may be presumed to be the 2,7 isomer since Bruce and Thomson (1955) reported that the 2,6 isomer does not melt below 200°; this statement agrees with the mp of 215° reported by Kuroda (1939) for his high-melting form. On paper chromatography in the ligroin-dimethylformamide system, the material of mp 125–126° behaved identically to the product from *M. caesia*, moving as a single red spot with an R_F value of 0.71. Furthermore, the ultraviolet spectra of the natural and the synthetic samples were identical.

For further study of the components of the red oil, gas chromatography proved very helpful. The samples were chromatographed on a column of SE-52 (183-cm coil, 3%) in a Chromolab instrument (Glowall Corp., Glenside, Pa.). The column was usually held at 180° and the carrier gas was argon at 20 lb pressure. In order to detect the naphthazarins and related naphthoquinones, it was necessary to operate the argon detector at a voltage of 1550; under these conditions, a nonlinear response was obtained. The sample of synthetic dimethylnaphthazarin showed a major peak with retention time of 5.0 minutes under these conditions, and very small traces of two other components were also present. A sample of 2-methylnaphthazarin[3] had a retention time of 3.2 minutes. Multiple peaks were obtained when various samples of the red, oily materials were injected—there were three major peaks with retention times of 1.6, 3.9, and 8.5 minutes, and at least seventeen other smaller peaks. Among these smaller peaks was one with a retention time of 5.0 minutes, corresponding exactly to the peak obtained with synthetic dimethylnaphthazarin under the same conditions. Mollisin itself was not eluted from these columns, even at a temperature of 230°. The general value of such a column was suggested by the observation that simpler naphthoquinones, e.g., menadione, could be successfully chromatographed at a lower temperature such as 150°.

Further evidence for the presence of the 2,7-dimethylnaphthazarin was provided by a study of the *O*-trimethylsilyl derivatives. For this purpose, small amounts of the synthetic and natural products were dissolved in 0.2 ml of acetonitrile. After the addition of a few drops of bis-trimethylsilylacetamide[4] and refluxing for about 0.5 minute, the now yellow solutions were chromatographed. From the synthetic 2,7-dimethylnaphthazarin, a major peak with retention time of 15 minutes was obtained; a small peak with retention time of 0.9 minute was also observed. The derivative from 2-methylnaphthazarin had a retention time of 11.1 minutes. From the red oil obtained from the mold, two major peaks were present with retention times of 1.6 and 3.8 minutes. About eighteen other peaks were also observed, and one of these coincided exactly with the position of the major peak from 2,7-dimethylnaphthazarin itself. This peak with a retention time of 15 minutes was not observed in the chromatogram of the red oil prior to trimethylsilylation.

Discussion

Although tracer studies of biosynthetic processes are generally carried out in liquid medium, excellent utilization of the added radioactive precursors was obtained in these experiments with mold colonies on solid agar media. The results establish a fundamental role for acetate in mollisin biosynthesis and probably of malonate as well. From carboxyl-labeled acetate or malonate, ^{14}C was definitely present at C-2 and C-7, while C-11 and C-12 were not labeled. The total activity in the Kuhn-Roth acetic acid was 33.8% of that of mollisin with [1-^{14}C]acetate and 32.8% of that of mollisin with [1,3-^{14}C]malonate. These values agree well with that expected if the acetate contains two of a total of six labeled atoms in mollisin (33.3%). Furthermore, the activities of the Kuhn-Roth CO_2 samples agree with that to be expected for four labeled atoms. It is therefore likely that activity is also present at C-4, 5, 9, and 13 in these experiments in an alternating pattern. The unambiguous observation of activity at C-2 and C-7 from the carboxyl-labeled acetate and malonate is that expected from the "polyacetate" theory. Under our experimental conditions designed to give optimum incorporation of isotope, malonate (or malonyl CoA) was apparently converted entirely to acetate (or acetyl CoA) prior to incorporation. It was therefore impossible to demonstrate any specific "starter" acetate units in the mollisin biosynthesis.

The only other naphthoquinone compound from molds which has been studied, javanicin, is also derived by the acetate plus polymalonate pathway (Gatenbeck and Bentley, 1965). It seems likely that other simple naphthoquinones will be synthesized in this way so that the steps leading to benzoquinones, naphthoquinones, and anthraquinones in molds are generally similar.

Since in the mollisin structure[5] there are two methyl groups attached to the naphthoquinone nucleus, it follows that if the nucleus and dichloroacetyl side chain were derived from a *single* acetate plus polymalonate chain, at least one methyl group would have to be added by a C_1 addition. The results of the methionine experiments, however, rule out this possibility. Another pathway, suggested by Thomas,[6] was that a single

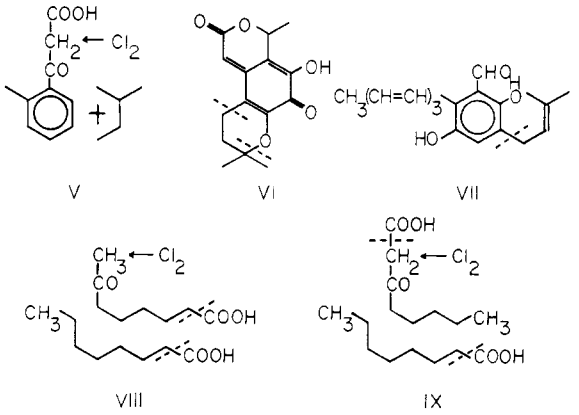
[3] 2-Methylnaphthazarin was generously provided by Professor R. H. Thomson.

[4] We are grateful to Dr. J. Klebbe for this material.

[5] The new structure for mollisin, III, was confirmed by nuclear magnetic resonance spectroscopy, kindly carried out by Dr. J. R. Dyer. In particular, absorptions at 3.21 τ and 2.84 τ were in agreement with two protons attached to the quinoid nucleus, rather than one as required by compound II.

[6] R. Thomas, personal communication.

acetate plus polymalonate chain might yield a benzene precursor containing one methyl and the dichloroacetyl group, and a subsequent addition of a C_5 mevalonate-derived unit would then give the second ring and the second C-methyl group (compound V, Scheme 3). An analogy to this was the derivation of the dimethylpyran ring of fuscin (compound VI, Scheme 3)



SCHEME 3

from mevalonic acid; similarly in the formation of auroglaucin (compound VII, Scheme 3), the addition of a C_5 unit to a benzenoid precursor is known to take place (Birch, 1960). Similar ideas have been expressed with regard to the biosynthesis of plant naphthoquinones, such as plumbagin and droserone (Robinson, 1955) and chimaphilin (2,7-dimethylnaphthoquinone) (Thomson, 1962). However, in three experiments with [2-^{14}C]mevalonic acid (added either as the salt or lactone), no incorporation of activity into mollisin was observed.

The only remaining possibility is that more than one acetate plus polymalonate chains are involved. Although a relatively uncommon situation, it has been shown by Gatenbeck and Mosbach (1963) that two chains are used in the biosynthesis of citromycetin; furthermore, the participation of an acetoacetyl unit as a second chain has been described in rotiorin biosynthesis by Holker *et al.* (1964). In the case of mollisin biosynthesis, the two most likely possibilities are those indicated as compounds VIII and IX (Scheme 3). Of these, one (compound VIII) involves the chlorination of a terminal methyl group, the other (compound IX), the chlorination of a β-keto acid and a decarboxylation. In studies of the chlorination step in caldariomycin formation, it was observed by Beckwith and Hager (1963) that the chloroperoxidase system catalyzes the chlorination of β-diketones and β-keto acids. In view of this observation, it seems likely that a similar situation is the case in *M. caesia*. The chlorination is therefore postulated as shown in compound IX (Scheme 3); both methyl groups in this case are derived from "starter" acetate units. The alternate possibility that a discrete dichloroacetate unit is added to some precursor seems unlikely in view of the failure of *M. caesia* to use either labeled monochloroacetic acid or monobromoacetic acid for mollisin production. The dichloroacetyl group of mollisin is of considerable interest since this group is found but rarely in naturally occurring compounds. In the case of one such product, chloramphenicol, the dichloroacetyl unit itself may be a biosynthetic precursor, since in this case the group is attached to a nitrogen rather than a carbon atom; it is also known that acetate itself is incorporated rather specifically into this unit (Gottlieb *et al.*, 1962).

It was of interest that *M. caesia* did not utilize NaBr for the production of a bromo mollisin analog. In fact, the presence of bromide prevented the normal production of mollisin itself. A similar situation has been observed in studies of tetracycline biosynthesis. In the latter case, some *Streptomyces* strains use bromide to synthesize a bromo compound (halide scavengers) while others did not so use bromide and are sensitive to inhibition of chloride utilization by bromide (Doerschuk *et al.*, 1959).

In view of the mild conditions used in isolation of the *Mollisia* metabolites (chloroform extraction, low-temperature sublimation), it seems unlikely that the dimethylnaphthazarin which we isolated was formed as an artifact of isolation. It may, however, represent a physiological degradation of mollisin itself, brought about by elimination of the dichloroacetyl group and the introduction of an OH group. Although naphthazarins are not found frequently as natural products, the plant product hydroxyserone (3-hydroxy-2-methylnaphthazarin) is rather similar to the product isolated by us. Another plant naphthoquinone, chimaphilin (2,7-dimethylnaphthoquinone), is of further interest in having two methyl groups located analogously to those of mollisin and the dimethylnaphthazarin.

Acknowledgment

We are very much indebted to Dr. Overeem for sending a copy of his dissertation (Overeem, 1962) prior to the publication of the revised structure of mollisin. Dr. Overeem also kindly supplied a sample of mollisin.

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Synthesis of 2-Solanesyl-1,4-naphthoquinone, New Member of a Vitamin K_2 Group*,†

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ABSTRACT: 2-Solanesyl-1,4-naphthoquinone and 2-phytyl-1,4-naphthoquinone have been synthesized by the reaction of solanesol and phytol, respectively, with the 1,4-naphthohydroquinone, and have been extensively characterized by spectral and chromatographic data. The 2-solanesyl-1,4-naphthoquinone appears to correspond in ultraviolet absorption characteristics to the described quinone, "SFQ," from *Streptococcus faecalis* 10C1, although the eight-unit isoprenolog is not excluded. The 2-phytyl-1,4-naphthoquinone and "SFQ" appear to be different but related compounds; chromatographic data also support these interpretations. The 3-desmethyl forms of vitamin K_2, which are in nature, are of particular interest in differentiating the biochemical functions of various quinones; the 3-methyl group of the better known methyl homologs is essential to the proposed quinone-methine mechanism of oxidative phosphorylation.

2-Solanesyl-1,4-naphthoquinone (compound I) has been synthesized as a new member of a new group of naphthoquinones which has recently been found to occur naturally and which has been considered as a group of "2-desmethyl vitamin K_2's." A new naphthoquinone from *Streptococcus faecalis* 10C1 has been isolated (Baum and Dolin, 1963b). This quinone, SFQ,[1] was the only one detected in the fermentations of *S. faecalis*. The limited availability of the quinone by isolation did not permit a definitive structural determination of the new quinone. However, it was apparent that the properties of SFQ were consistent with those of a 1,4-naphthoquinone substituted in the 2- position with a 40- to 45-carbon β-alkenyl side chain (compound II); later it was found that synthetic 2-phytyl-1,4-naphthoquinone (having a 20-carbon side chain) is "virtually identical with SFQ" in respect to absorption-peak positions in the ultraviolet spectrum. Through the generosity of Dr. Boyd H. Woodruff of the Merck, Sharp and Dohme Research Laboratories, Rahway, N.J., cells of *S. faecalis* (MB-130) were obtained and extracted. However, the cells of this strain did not yield any such quinone by the isolation techniques which were used, and which were based on those described for *S. faecalis* 10C1 (Baum and Dolin, 1963b).

Three naphthoquinones have more recently been reported (Lester *et al.*, 1964) from *Hemophilus parainfluenzae*. These three compounds were termed "2-desmethyl vitamin K_2's." The principal component appeared to have a C_{30} isoprenoid side chain (compound III) and there were lesser amounts of possibly the C_{25} and C_{35} isoprenologs (compounds IV and V).

A quinone has been isolated from teakwood (*Tectona grandis* L.) which apparently is the cause of marked skin irritation and eczema that results when workers

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[1] Abbreviations used in this work: SFQ, substance purified from lipid extracts of a strain of *Streptococcus faecalis* (Baum and Dolin, 1963b); NMR, nuclear magnetic resonance.