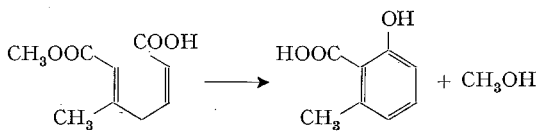
A Study on the Metabolism of Some Dienoic Compounds

Certain analogic aspects between the biogenetic cyclization of polyketonic chains to phenols[1] and the chemical synthesis of phenols from dienoic acids[2] has led us to study the metabolism of the latter in cultures of *Penicillium urticae*, a known source of phenolic compounds[3].

In particular we tried to ascertain if 6-methylsalicylic acid (an usual metabolite of the above micro-organism) could be formed from methyl 6-carboxy-3-methylhexa-2,5-dienoate[4-6] as in chemical synthesis[2]:

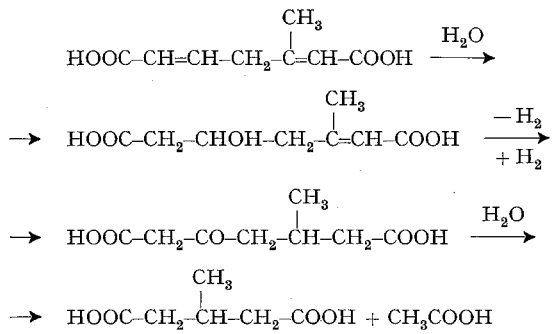


In our experiments we added a sterilized 0.5% aqueous solution of methyl 6-carboxy-3-methylhexa-2,5-dienoate to sterilized portions of modified Czapeck's nutrient medium[7] containing an inoculum of *P. urticae* Bainier, CBS NRRL 909. The cultures were kept for 10 days at 20 °C.

Mycelium developed normally if the amount of added monoester was less than 0.2 g/100 g of Czapeck's medium. When no methyl carboxyhexadienoate had been added, exhaustive extraction with ethyl ether of the clear solution filtered from the fermentation mash gave 0.1 g of organic acids. Thin layer chromatography of this mixture showed the presence of gentisic acid.

No significant radioactivity was found in gentisic acid or in 6-methyl salicylic acid when the fermentation was run in the presence of carboxymethyl-hexadienoic ester ^{14}C labelled in 5 and 6 positions. 3-methylglutaric acid was formed instead, the formation of acids from normal metabolism being inhibited. As few as 0.26 g of monoester added to 160 ml of Czapeck's nutrient medium sufficed to prevent almost completely the formation of normal metabolic compounds and gave 0.07 g of 3-methylglutaric acid. The original monoester was recovered unchanged or in the form of the free acid, partially transformed into its 3,5 and 2,4 isomers (0.18 g). A larger amount of monoester prevented the growth of the mycelium.

3-methylglutaric acid is likely to originate by hydration and oxidation of one double bond, followed by splitting off of acetic acid and hydrogenation of the other double bond:



This behaviour was not observed in the case of straight chain compounds such as methyl 6-carboxyhexa-2,5-dienoate, which underwent complete degradation. Apparently the compound is hydroxylated, oxidized and degraded, and its fragments (acetic acid) are used for normal metabolism.

Conjugated acids (sorbic acid, 3-methyl-3,5-heptadiendioic acid, etc.) inhibited the fermentation and were recovered unchanged. Other straight or branched chain acids could not be studied because, even in small amounts, they prevented the growth of the mycelium.

Similar experiments were run on *Aspergillus niger*, but in no case was the formation of phenolic acids observed; 3-methylglutaric acid was always obtained in an amount 50% lower than in *P. urticae* tests. Methyl 6-carboxy-3-methylhexa-2,5-dienoate and the acids from its saponification (3-methylhepta-3,5-dien-1,7-dioic acid; 3-methyl-2,5-dien-1,7-dioic acid, and 3-methylhepta-2,4-dien-1,7-dioic acid) were recovered in very small amounts. This means that *A. niger* is capable of breaking the molecule of the above compounds using the fragments for its normal metabolism.

We can conclude that the metabolism of 2,5-dienoic acids seems to follow a path either leading to the oxidative degradation or to the formation of stable products (generally containing conjugated double bonds) which inhibit the fermentation. In the case of 6-carboxy-3-methyl-2,5-hexadienoic acid the hydrogenation of the double bond bearing the methyl group seems to utilize the hydrogen furnished by the dehydrogenation of the hydration product of the other double bond[8].

Riassunto. Acidi dienoici marcati con ^{14}C facilmente aromatizzabili a fenoli per via chimica, sono stati somministrati a muffe produttrici di fenoli. Anzichè l'aromatizzazione è stata osservata una degradazione, che nel caso dell'acido 3-metilepta-2,5-dien-1,7-dioico conduce all'acido 2-metilglutarico.

G. AGNÈS[9], G. P. CHIUSOLI[9] and A. QUILICO[10]

Centro di Chimica Organica Esplorativa, Montecatini-Edison, Novara (Italy) and Istituto di Chimica del Politecnico di Milano, Milano (Italy), 12 May 1969.

[1] A. J. BIRCH, Proc. natn. Acad. Sci. *1962*, 4. – G. BRAM, Tetrahedron Letters *41*, 4069 (1967).
[2] G. P. CHIUSOLI and G. AGNÈS, Chimica ind. appl. *46*, 25 (1964); G. AGNÈS and G. P. CHIUSOLI, Chimica ind. appl. *49*, 465 (1967).
[3] E. W. BASSETT and S. W. TANNENBAUM, Experientia *14*, 38 (1958).
[4] The monoester was used instead of the free dicarboxylic acid for its easier preparation.
[5] This product was prepared by Dr. L. CASSAR from methyl 4-bromocrotonic acid, acetylene and CO. The preparation of the monoester ^{14}C labelled in 5 and 6 positions (from labelled acetylene) and the measurement of the radioactivity were made by Prof. M. DUBINI and Dr. G. P. VICARIO.
[6] L. CASSAR and G. P. CHIUSOLI, Chimica ind. appl. *48*, 323 (1966).
[7] The modified Czapeck's nutritive medium was prepared by dissolving 40 g of glucose, 4.6 g of ammonium tartrate, 1.02 g of KH_2PO_4, 0.51 g of KCl; 0.51 g of $MgSO_4 \cdot 7H_2O$ and 0.01 g of $FeSO_4 \cdot 7H_2O$ in 1 l of water.
[8] Acknowledgment. The authors are indebted to Dr. J. J. ELLIS of the N.R.R.L., Peoria (USA) for having kindly supplied a culture of *P. urticae*.
[9] Centro di Chimica Organica Esplorativa, Montecatini-Edison, Via del Laboro 4, Novara (Italy).
[10] Istituto di Chimica del Politecnico di Milano (Italy).