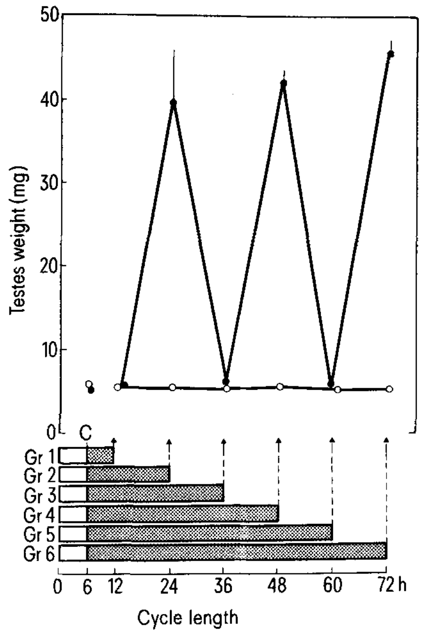


Testicular responses of yellow-throated sparrows under resonance light-dark cycles after 30 days of transfer to long day (15L:9D). Open bar is 6 h basic photophase. Stippled bars represent longer duration of scotophase. C is control group. Open and solid circles represent the initial and final testes weights. Gr represents groups. Vertical bars represent the statistical error if it exceeds the limits of the point symbol. Seven birds, one from each group, died at the end of the experiment. Data from dead birds were excluded in our analysis.

The results are in agreement with those obtained for the white-crowned sparrow and golden-crowned sparrow[5], Redheaded Bunting[7] and Blackheaded Bunting[8] (although in the latter, 72 h cycle was not investigated). Further, in the white-crowned sparrow a 16 (8L:8D) h cycle dissipated the refractory period, although it maintained it in the golden-crowned sparrow[5], but the reason for that is unknown. However, our results suggest that an endogenous circadian rhythm is involved in photoperiodic time measurement during the termination of the refractory period in *G. xanthocollis*. Thus, the results can be interpreted on the basis of an 'external-coincidence' model (Bünning hypothesis), which suggests that the termination of the refractory period depends on the non-coincidence of light (daily or otherwise) with an endogenous circadian rhythm of photosensitivity[11,12].

1 This investigation was supported by a UGC grant to PDT and BHU to PMT.
2 To whom reprint requests should be addressed.
3 Hamner, W. M., Ecology *49* (1968) 211.
4 Murton, R. K., Lofts, B., and Westwood, N. J., Gen. comp. Endocr. *14* (1970) 107.
5 Turek, F. W., Science *178* (1972) 1112.
6 Sansum, E. L., and King, J. R., J. comp. Physiol. *98* (1975) 183.
7 Prasad, B. N., and Tewary, P. D., Physiol. Zool. *56* (1983) 64.
8 Tewary, P. D., and Kumar, V., Condor *86* (1984) 27.
9 Nanda, K. R., and Hamner, K. C., Bot. Gaz. *120* (1958) 14.
10 Whistler, H., in: Popular handbook of Indian birds, p. 224. Gurney and Jackson, London, Edinburgh 1928.
11 Farner, D. S., Am. Zool. *15*, suppl. (1975) 117.
12 Turek, F. W., in: Diurnal rhythms and the seasonal reproductive cycle in birds, p. 144. Eds I. Assenmacher and D. S. Farner. Environmental endocrinology, Springer, Berlin, Heidelberg, New York 1978.

0014-4754/85/101350-02$1.50 + 0.20/0

Anaerobic microbial conversion of three hydroxycinnamic acids

M. Nali, B. Rindone, S. Tollari, V. Andreoni and V. Treccani

Istituto di Chimica Organica dell'Università, Via Venezian 21, and Istituto di Microbiologia Agraria e Tecnica dell'Università, Via Celoria, I-20133 Milano (Italy), 27 September 1984

Summary. Caffeic, ferulic and synapic acid are anaerobically degraded by a microbial consortium via the reduction of the double bond of the side chain, the replacement of the nuclear hydroxyl group in position 4 by hydrogen, the demolition of the side chain by the loss of a C_1 unit.

Key words. Anaerobic metabolism; phenylpropanoid catabolism; methanogenic consortium; metabolic sequence; phenolic acid degradation.

Phenolic acids frequently occur in plants as hydroxycinnamic acids ester-linked to polysaccarides and large amounts of simple phenolic acids, such as ferulic (**1**), p-coumaric (**2**) and syringic acids (**3**), are released during the aerobic catabolism of lignin[1]. The aerobic catabolism of these aromatic substances has been largely studied[2,3]; on the contrary the anaerobic degradation of the simplest aromatic lignin derivatives has not received much attention. Healy et al.[4] observed that ferulic acid (**1**) is biodegradable to methane and carbon dioxide under strict anaerobic conditions by a methanogenic consortium of bacteria through initial ring reduction followed by ring cleavage. Kaiser and Hanselmann[5] reported that syringic acid (**3**) is completely degraded to methane and carbon dioxide via the intermediate formation of gallic acid (**4**) by two demethylation steps.

Our attention was focused on the reactions involved in the early steps of the anaerobic pathway of different hydroxycinnamic acids by the bacterial community from an anaerobic sewage sludge.

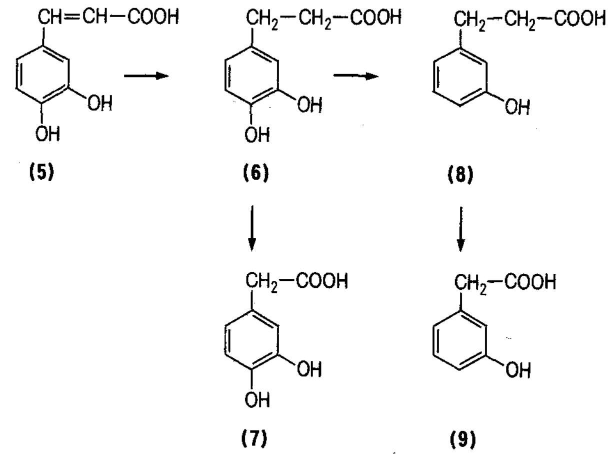
Anaerobic enrichment cultures of this bacterial community were obtained in prereduced mineral medium FW[6] added of 2‰ (w/v) trypticase, 2‰ (w/v) peptone and 0.5‰ (w/v) caffeic acid (**5**), dispensed into rubber sealed bottles. Anaerobic conditions were assured by flushing the bottles with a mixture of hydrogen and carbon dioxide (80:20). Resazzurrin was added at a final concentration of 0.001% as a redox indicator. Unter these conditions the disappearance of the UV absorption spectrum of caffeic acid (**5**) was observed after 20 days of incubation.

When prereduced mineral medium FW, in which caffeic acid (**5**) was the only carbon and energy source, was inoculated with the consortium caffeic acid (**5**) (scheme 1) was transformed after a 5-day incubation into 3,4-dihydroxyphenylpropionic acid (**6**); 3,4-dihydroxyphenylacetic acid (**7**) and 3,4-dihydroxyphenylpropionic acid (**8**). After a 30-day incubation compounds (**6**) and (**8**) were still present, together with 3-hydroxyphenylacetic acid (**9**).

All these compounds were detected by GLC-MS after diazomethane treatment of the crude extract at pH = 2 and were identified by comparison with authentical specimens.

Since the appearance of some metabolites seemed to depend from the incubation time, a study related to the sequence of these

Scheme 1



transformations was carried on monitoring the amount of metabolites vs time. The quantitative analyses were performed by GLC of the samples after diazomethane treatment using a 5% OV 101 stainless steel column 50 cm × $\frac{1}{8}$ in., column temp. 150–220 °C, 35 °C/min; inj. temp. 250 °C; flame ionisation temp. 250 °C; carrier gas nitrogen 25 ml/min, using the alkaloid (**10**) as internal standard.

Figure 1 shows the rapid decrease with time of the concentration of caffeic acid and the appearance of a high concentration of its dihydroderivative, 3,4-dihydroxyphenylpropionic acid (**6**), deriving from the reduction of the double bond of the side chain. In addition compound (**6**) was transformed by the same consortium to compounds (**7**), (**8**) and (**9**).

Significant amounts of the nuclear dehydroxylated product (**8**) appeared in the quantitative analysis, whereas compound (**7**), qualitatively detected after 5 days, was not found in significant amounts in quantitative assays.

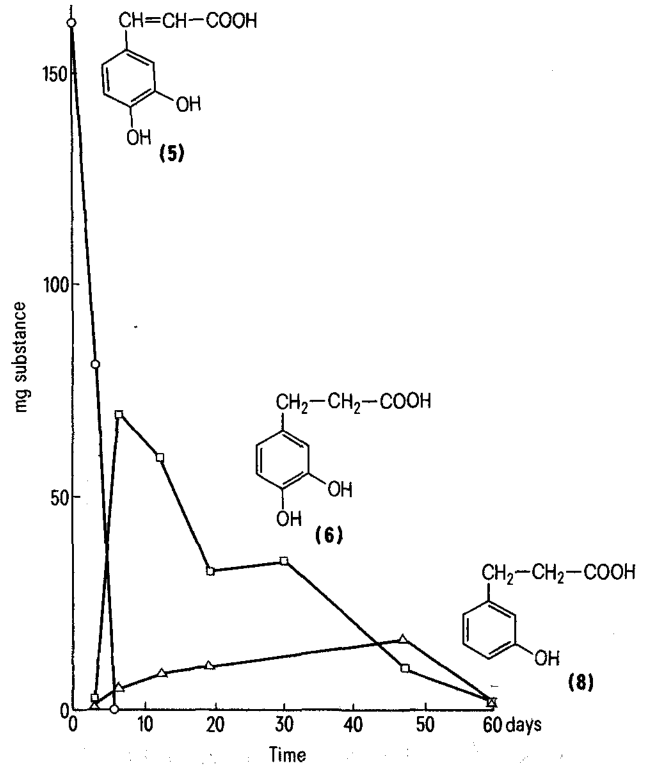


Figure 1. Time course of the anaerobic metabolism of caffeic acid.

The caffeic acid (**5**)-adapted bacterial community was also able to utilize ferulic acid (**1**) and synapic acid (**11**) as the only carbon and energy source.

Ferulic acid (**1**) (scheme 2) was transformed by the consortium giving, after a 9-day incubation, 3-methoxy-4-hydroxyphenylpropionic acid (**12**); 3-methoxyphenylpropionic acid (**13**); 3-methoxyphenylacetic acid (**14**) and 3-methoxy-4-hydroxyphenylacetic acid (**15**). After a 24-day incubation compounds (**12**), (**13**) and (**14**) were detected by glc-ms after diazoethane treatment of the crude extract at pH = 2 and identified by comparison with authentical specimens.

Scheme 2

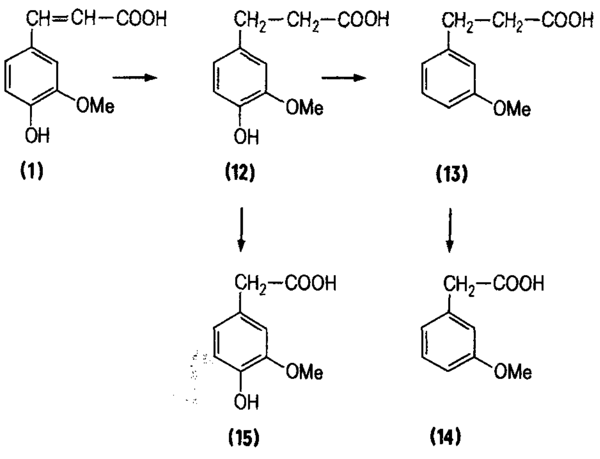


Figure 2 shows the amount of metabolites vs time, indicating that ferulic acid was rapidly transformed into its dihydroderivative (**12**), as already noted in caffeic acid degradation. Moreover, both compound (**15**), deriving from the loss of a C_1 unit from the side chain, and compound (**13**), deriving from the

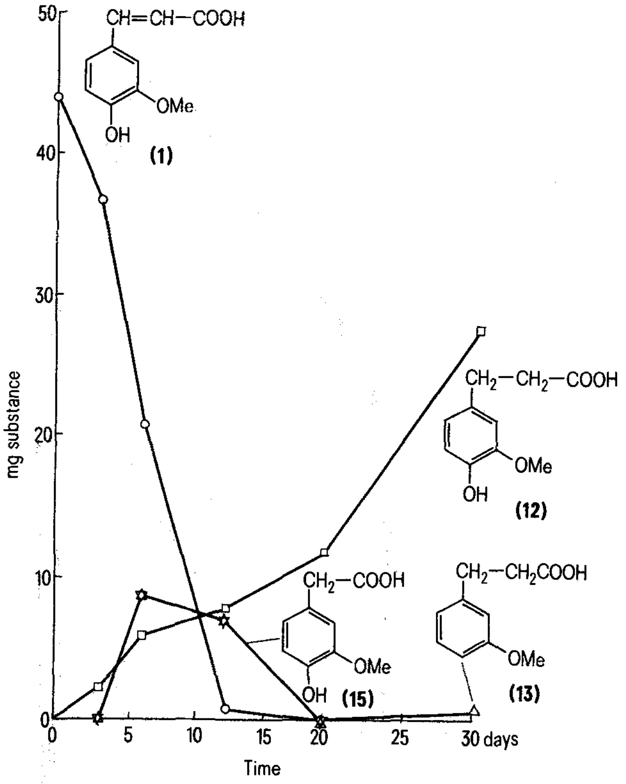
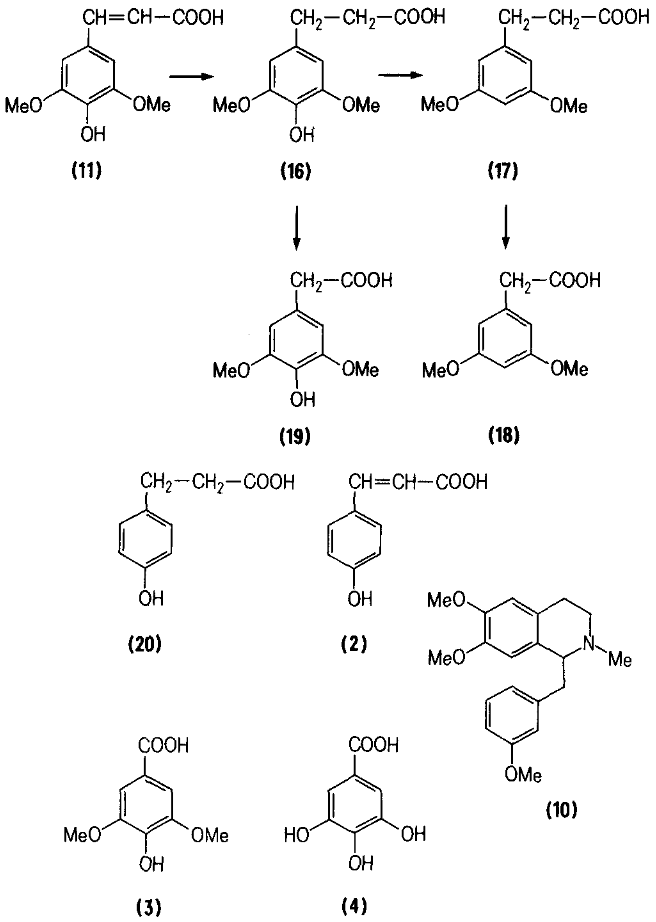


Figure 2. Time course of the anaerobic metabolism of ferulic acid.

loss of a C_1 unit from the side chain, and compound (**13**), deriving from a nuclear dehydroxylation reaction, appear in similar amounts up to 19 days.

Synapic acid (**11**) (scheme 3) was transformed after a 9-day incubation into 3,5-dimethoxy-4-hydroxyphenylpropionic acid (**16**); 3,5-dimethoxyphenylpropionic acid (**17**); 3,5-dimethoxyphenylacetic acid (**18**) and 3,5-dimethoxy-4-hydroxyphenylacetic acid (**19**). After a 24-day incubation compounds (**16**), (**17**) and (**18**) were detected.

Scheme 3



A similar anaerobic transformation was shown to occur also incubating 4-hydroxy phenylpropionic acid (**20**) and p-coumaric acid (**2**) for 12 days with the consortium.

From these experimental data it seems that three reactions are involved in the anaerobic catabolism of the tested hydroxycinnamic acids:

1) The reduction of the double bond of the side chain;

2) the replacement of the nuclear hydroxyl group in position 4 by hydrogen;

3) the demolition of the side chain by the loss of a C_1 unit.

Ethylbenzenes and styrenes deriving from the direct decarboxylation of the side chain were never observed.

The data shown in figure 1 for caffeic acid suggest that compound (**7**), formed by a C_1 unit loss from the side chain, is rapidly dehydroxylated to compound (**9**), and that compound (**8**), formed by a dehydroxylation reaction, does not undergo further degradation by a C_1 loss from the side chain at a comparable rate. Since compounds (**7**) and (**8**) derive from the common intermediate (**6**), it can be concluded that the nuclear dehydroxylation reaction of caffeic acid, carried on by our adapted consortium, is more rapid than the C_1 loss from the side chain.

The presence of the meta hydroxylated compounds (**8**) and (**9**) suggests that the dehydroxylation reaction occurs mainly at the para position.

Acknowledgments. This work was supported by a grant from the Consiglio Nazionale delle Ricerche. We thank Dr Michela Villa for her collaboration in the experimental work.

1 Kuwahara, M., Metabolism of lignin-related compounds by bacteria in lignin biodegradation: Microbiology, Chemistry and Potential Applications, vol. 2, p. 146. CRC Press, New York 1980.
2 Toms, A., and Wood, J. M., Biochemistry *9* (1970) 337.
3 Blakley, E. R., and Simpson, F., Can. J. Microbiol. *10* (1964) 175.
4 Healy, J. B. Jr, Young, L. Y., and Reinhard, M., Appl. envir. Microbiol. *39* (1980) 436.
5 Kaiser, J. P., and Hanselmann, K. W., Archs Microbiol. *133* (1982) 185.
6 Ferry, J. H., and Wolfe, R. S., Archs Microbiol. *107* (1976) 33.

0014-4754/85/101351-03$1.50 + 0.20/0

5-(Hydroxyimino)-4-methoxy-2-(pivaloylimino)thiazolidine-3-acetamide, a reduced nitroheterocyclic derivative with potent schistosomicidal properties

P. J. Islip and M. V. Bogunović-Batchelor[1]

Medicinal Chemistry Laboratories, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS (England),
23 August 1984

Summary. The synthesis and antischistosome properties of 5-(hydroxyimino)-4-methoxy-2-(pivaloylimino)thiazolidine-3-acetamide (**1**) are described. The compound was prepared by reduction of the nitrothiazoline (**2**) with stannous chloride in methanol, and represents the first example of a reduced nitroheterocyclic compound showing potent schistosomicidal properties. The possible relationship of compounds such as **1** to the as yet unidentified reduced active but toxic entities formed in vivo from nitroheterocyclics like metronidazole is discussed.

Key words. Metronidazole; methoxy-oximinothiazolidine; reduced nitroheterocyclic; schistosomicidal; *Schistosoma mansoni.*

A range of nitroheterocyclic and nitroaromatic compounds have been shown to possess antischistosome properties[2], and indeed several clinically used drugs of this type have emerged with good efficacy against human *Schistosoma* infections[2b]. In addition, these and many other nitro compounds are variously active against a wide variety of helminths, protozoa, fungi and bacteria.

Although many investigations have been made into the mode of action of antiparasitic nitroheterocyclics, the precise identity of the active entity has yet to be established. Nevertheless, to date, all derivatives of the active nitro compounds in which the nitro group has been reduced appear to be inactive in the screens where the parent nitro compound is active.

Extensive earlier investigations have shown that certain 5-nitro-2-thiazolines possess extremely potent schistosomicidal properties in both rodents and primates[3]. We wish to report here that