Biosynthesis of Methanofuran

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The formation of the furan ring of methanofuran can be explained on the basis of an aldol condensation between dihydroxyacetone phosphate and pyruvate, and the 4,5-dicarboxyoctanedioic acid moiety of the coenzyme appears to be assembled from α-ketoglutarate, acetate, and pyruvate.

Methanofuran serves as the primary CO_2 acceptor in methanogenic bacteria, and its structure has been established recently (Figure 1).[1,2] We have studied the biosynthesis of methanofuran by incorporation experiments with ^{13}C-labelled acetates in *Methanobacterium thermoautotrophicum* (Marburg strain). Briefly, the micro-organism was grown in mineral medium containing 4 mM of ^{13}C-labelled acetate in an atmosphere of CO_2/H_2 (1 : 4, v/v). At the end of the logarithmic growth phase the cells were harvested, and methanofuran was isolated from the cell extract. Hydrolysis of cellular RNA yielded purine nucleosides which were isolated chromatographically. Acid hydrolysis of cell protein yielded amino acids which were also isolated chromatographically.

^{13}C Enrichments in the isolated metabolites were determined by ^{13}C n.m.r. spectroscopy. Methanofuran was measured in D_2O at an apparent pH of 3.7. The ^1H n.m.r. spectrum was assigned on the basis of COSY and Hartmann–Hahn[3] experiments. The ^{13}C n.m.r. signals were subsequently assigned on the basis of DEPT, ^1H–^{13}C chemical shift correlation, and inverse COLOC[4] measurements. The analysis of ^{13}C–^{13}C coupling in methanofuran biosynthetically derived from [1,2-$^{13}C_2$]acetate by ^{13}C-COSY and INADEQUATE experiments gave additional confirmation of signal assignments. We could not yet establish which of the carboxyl groups (C-1e or C-8e) participates in the amide bond. Relative ^{13}C abundances were calculated for each respective carbon atom by comparison of the ^{13}C n.m.r. spectra of labelled methanofuran with natural abundance material (Table 1).

The 4-(hydroxymethyl)furfurylamine moiety shows unequivocal evidence for the incorporation of two intact acetate units (Scheme 1). This is most clearly seen in the experiment with [1,2-$^{13}C_2$]acetate, where we observe coupling between C-2a and C-3a ($^1J_{CC}$ 73.7 Hz) and between C-4a and C-7a ($^1J_{CC}$ 55.1 Hz), but no coupling between C-3a and C-4a.

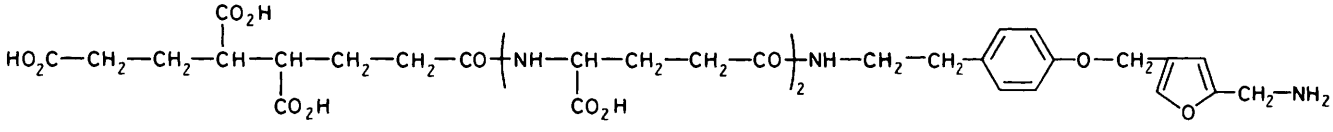
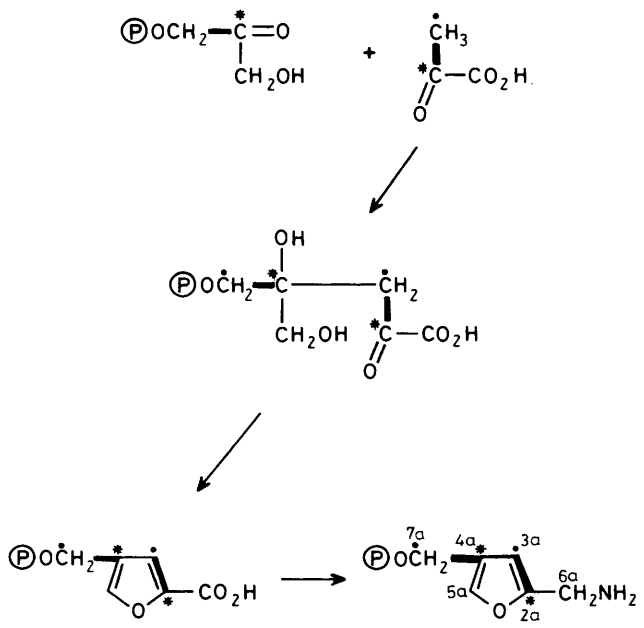


Figure 1. Structure of methanofuran.

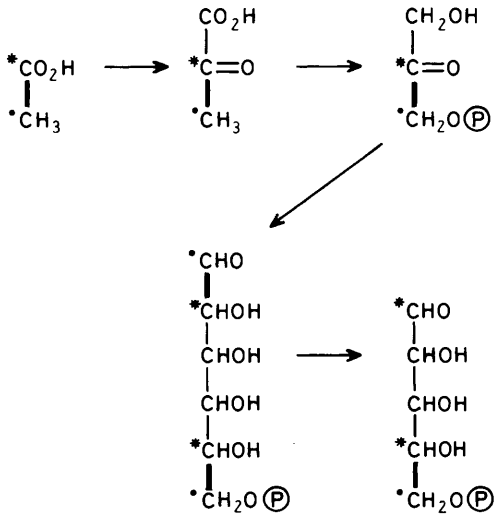
Table 1. ^{13}C-Enrichments of methanofuran samples from cultures of *M. thermoautotrophicum* supplemented with ^{13}C-labelled acetate. Measurements were performed in D_2O/0.8 M phosphate buffer, pH 3.7.

Position	^{13}C Chemical shift, δ	Relative ^{13}C enrichment, % [1-^{13}C]-Acetate	[2-^{13}C]-Acetate	[1,2-$^{13}C_2$]Acetate ($^1J_{CC}$/Hz)
2a	149.4	5.7	1.3	5.2 (73.5)
3a	113.5	1.3	4.7	4.2 (73.8)
4a	124.2	4.0	1.0	4.3 (55.5)
5a	145.2	1.4	1.1	1.0
6a	37.9	1.7	1.2	1.2
7a	64.0	1.5	4.9	5.0 (54.8)
1b	158.2	1.6	1.4	1.2
2b/6b	117.4	3.1	1.2	4.4
3b/5b	132.3	1.0	7.6	9.3
4b	134.6	3.4	1.1	8.8
7b	35.8	1.3	7.4	ND[a] (35.7)
8b	42.7	6.4	1.2	8.8 (34.8)
1c/1d	178.8	1.0	1.4	1.2
5c/5d	177.1	1.5	1.2	1.2
2c/2d	55.4	1.1	1.1	1.2
	55.3	1.1	1.3	1.2
3c/3d	29.6	2.3	3.6	ND[a] (35.1)
	29.3	3.2	4.2	ND[a] (35.5)
4c/4d	34.3	2.2	3.6	ND[a] (34.5)
	34.0	2.6	4.3	ND[a] (34.0)
9e/10e	180.9	ND[a]	1.3	6.1 (53.1)
	180.85	ND[a]	1.3	1.2
8e	179.8	1.0	1.6	1.2
1e	177.0	1.2	1.2	1.2
4e/5e	50.45	1.0	ND[a]	1.2
	50.35	1.0	ND[a]	6.1 (53.1)
2e/7e	35.5	2.7	4.4	ND[a] (36.0)
	34.1	4.0	1.6	ND[a] (35.5)
3e/6e	28.1	2.3	4.5	7.9 (34.4)
	27.4	1.0	7.6	7.6 (35.1)

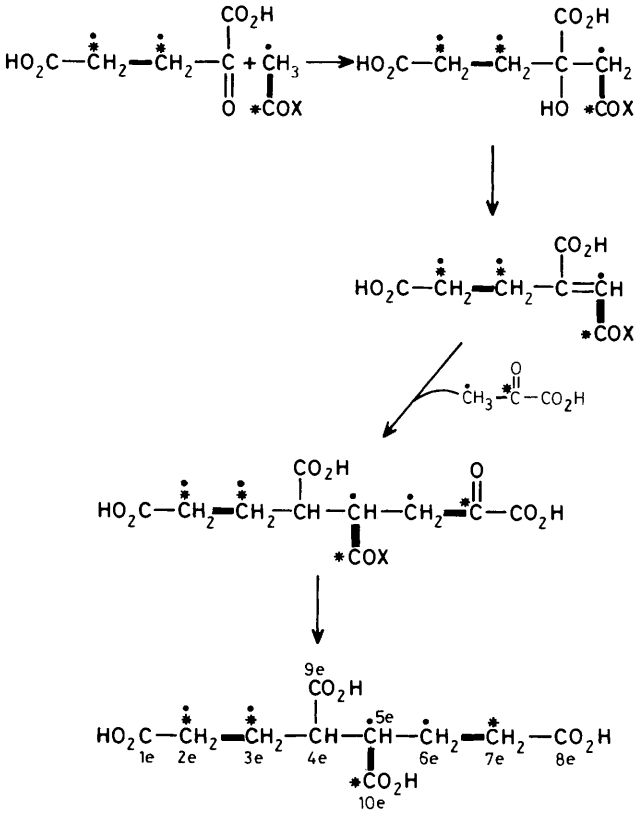
[a] ND, not determined, owing to signal overlap.



Scheme 1. Hypothetical mechanism for the biosynthesis of the furan moiety of methanofuran. Positions labelled from the carboxy group (*) and the methyl group (•) of acetate are indicated. Bars indicate pairs of carbon atoms jointly transferred from [1,2-$^{13}C_2$]acetate as shown by ^{13}C–^{13}C coupling.

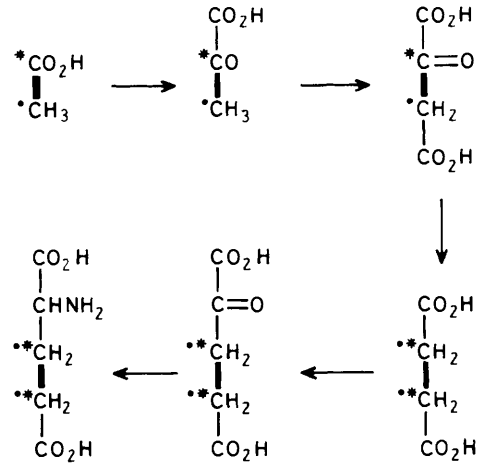


Scheme 2. Biosynthesis of carbohydrates in *M. thermoautotrophicum*. For details see Scheme 1.



Scheme 3. Hypothetical mechanism for the biosynthesis of the 4,5-dicarboxyoctanedioic acid subunit of methanofuran. For details see Scheme 1.

Little is known about the biosynthesis of furan systems. The substituted furan ring in the antibiotic reductiomycin is generated by ring opening of an aromatic amino acid as shown recently by Beale and Floss.[5] The observed labelling pattern of methanofuran shows clear evidence of a different mechanism, since formation of the furan ring *via* tyrosine would lead to coupling between C-3a and C-4a in the experiment with [1,2-$^{13}C_2$]acetate which was clearly not present.



Scheme 4. Biosynthesis of glutamate in *M. thermoautotrophicum*. For details see Scheme 1.

The observed labelling pattern can be explained by an aldol-type condensation between dihydroxyacetone phosphate and pyruvate (Scheme 1). Although the labelling pattern of dihyroxyacetone phosphate has not been observed directly, it can be easily inferred from the label distribution experimentally observed in the ribose moiety of guanosine isolated after hydrolysis of cellular RNA (Scheme 2).[6,7]

Three pairs of contiguous carbon atoms are incorporated from acetate into the 4,5-dicarboxyoctanedioic acid moiety of methanofuran (Scheme 3). The incorporation pattern observed for C-2e and C-3e is diagnostic of a glutarate derivative, most probably α-ketoglutarate, as shown by comparison with the labelling pattern of glutamate obtained from cell protein. It should be noted that the observed labelling pattern of glutamic acid is perfectly in line with the established biosynthetic pathway *via* reductive carboxylation (Scheme 4).[6,8,9] Carbon atoms C-5e and C-10e reflect the incorporation of an intact acetate moiety, and the labelling pattern of C-6e, C-7e, and C-8e appears consistent with the incorporation of pyruvate. A hypothetical mechanism for the formation of the 4,5-dicarboxyoctanedioic acid moiety is summarized in Scheme 3.

The biosynthesis of the 4,5-dicarboxyoctanedioic acid moiety has been studied by White by mass spectroscopy.[10] White proposed the involvement of one molecule of α-ketoglutarate and two molecules of malonate as precursors. This suggestion is not consistent with our data.

It should be mentioned that the labelling patterns of the tyramine and glutamate moieties of methanofuran accurately reflect the labelling patterns of tyrosine and glutamate obtained by hydrolysis of cell protein.[6]

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