Biosynthesis of Zymonic Acid in *Trichosporon capitatum*

By J. L. BLOOMER* and M. A. GROSS

(*Department of Chemistry, Temple University, Philadelphia, Pennsylvania* 19122)

Summary Zymonic acid (**1**) is an artefact derived by decarboxylation from a natural metabolite [probably (**3**)], the biosynthesis of which from labelled precursors has been studied: malonic and methylmalonic acids are incorporated into different portions of the metabolite molecule, and from studies with labelled acetates and labelled glucoses, it is inferred that the metabolite arises from a derivative of methylmalonate and from a C_3 unit, probably tartronate (**4**) or a close derivative.

ZYMONIC ACID (**1**) has been isolated from the culture media of several different yeasts;[1] calculated on the dimethyl derivative (**2**), yields as high as 14% from glucose were reported. The structure (**1**) is based on a revised structure for (**2**) suggested by Haynes and Stanners.[2] Our n.m.r. measurements on (**2**) agree with this revised structure [τ 8·40 (3H, s), 6·38 (3H, s), 6·33 (3H, s), 5·80 (1H, s)]. We find, as suspected earlier,[3] that zymonic acid is an artefact from a true metabolite: culture filtrates made alkaline show no tetronate absorption at 265 nm, and acidification gives CO_2 in amount equimolar to the subsequently isolated (**2**). The precursor of zymonic acid, which we term protozymonic acid, is not a terminal metabolite since it was rapidly utilized if glucose concentration in the media fell below 2%.

Our biosynthetic studies on protozymonic acid suggest that it has structure (**3**) and that it is derived from a methylmalonate moiety and from a C_3 precursor, possibly tartronate or tartronic semialdehyde. ^{14}C-labelled substrates were added, at various stages of growth, to cultures of *T. capitatum* and the cultures were worked-up after 8 days. The dimethyl derivative (**2**) was prepared and was degraded (a) by Kuhn–Roth oxidation, giving C-2 and C-5 as acetic acid and the remainder of the molecule as CO_2, which was counted as $BaCO_3$ and (b) by lithium iodide–*s*-collidine decarboxylation,[4] giving C-6 as CO_2. (Until recently we were unable to obtain the other fragment in sufficient yield to be useful.) The large deviation in the Kuhn–Roth acetic acid + Kuhn–Roth CO_2 values is due to the extensive purifications of (**2**) required to reach constant specific activity giving rise to low activity/background ratios (the only exception to this being the [1-^{14}C]glucose run fed at 2 days).

not available at the time of the experiment, the 35% figure may well be too low.)

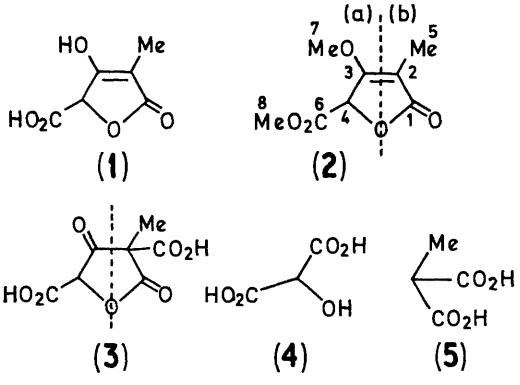
[1-^{14}C]Acetate, a potential precursor of malonate, also gave heavy labelling at C-6. [2-^{14}C]Acetate gave rise to minor but significant labelling at C-5, C-2, and C-6; this could arise through known metabolic pathways connecting [2-^{14}C]acetate with [2,3-^{14}C]succinate and [2,2′-^{14}C]methylmalonate, and with [1-^{14}C]acetate.

When [1-^{14}C]glucose was added at the beginning of a fermentation, the proportion of radioactivity at C-5 (45%) was greater than that (27%) obtained when the labelled sugar was added later. We attribute this effect to greater resynthesis, over the longer time, of glucose from triose phosphates, leading to "scrambling" of label between C-1 and C-6. If this is accepted it also follows that part (a) of the molecule is derived preferentially from C-1, C-2, and C-3 of glucose and not from the equilibrated triose phosphates of the normal Embden–Meyerhof pathway. The results

TABLE

Precursor	Administration time (days of fermentation)	Amount μCi	% Incorporation	% Total activity of (**2**) C-5	C-2	C-6	C-1,3,4,6—8 (as $BaCO_3$)
[1-^{14}C]Glucose	2	10	0·51	27	1	2	71
[3-^{14}C]Glucose	0	10	1·02	3	11	34	98
[6-^{14}C]Glucose	3	25	0·48	49	4	<1	54
[1-^{14}C]Glucose	0	25	0·34	45	0	0	49
[^{14}C]Methylmalonic acid ..	3	22	0·23	74	2	2	38
Diethyl [1,3-^{14}C]malonate ..	4	11	0·34	0	0	35	112
Sodium [1-^{14}C]acetate ..	4	55	0·47	<1	<1	47	92
Sodium [2-^{14}C]acetate ..	4	25	0·80	11	8	5	89

The results are summarized in the Table. [*Me*-^{14}C]Methionine gave very little radioactivity in (**2**), and none at C-5. [^{14}C]Methylmalonic acid gave (**2**) having the bulk of its radioactivity at C-5. With diethyl [1,3-^{14}C]malonate, 35% of the radioactivity was in C-6: if the malonate molecule is incorporated as a whole, another 35% must have been at C-3. (Since an appropriate cross check on this value was with [3-^{14}C] and [6-^{14}C]glucose are essentially consistent with this view (except that the decarboxylation result from [3-^{14}C]glucose, fed at the start of the fermentation, may be low in just the same manner as the malonate case described above, since the observed value in each case is 35% as compared with 50% predicted by our hypothesis. Tartronic acid (**4**) and tartronic semialdehyde are both thought to be derivable from C-1, C-2, and C-3 of glucose *via* non-Embden–Meyerhof mechanisms;[5,6] the oxidation state of tartronate corresponds to that of C-3, C-4, and C-6 in (**1**); and tartronate is potentially also derivable from malonate. On the other hand, the results indicate that part (b) of (**2**) could arise from triose. This route could lie through pyruvate, propionate, and methylmalonate, in agreement with the observed incorporation of the latter.



This investigation was supported (in part) by a U.S. Public Health Service Grant from the National Institute of Allergic and Infectious Diseases, The National Institutes of Health, National Science Foundation grants, and an institutional grant from the American Cancer Society. One of us (M.A.G.) thanks Temple University for a research fellowship.

(*Received, September 25th*, 1969; *Com.* 1454.)

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