A NEW INTERMEDIATE IN NAPHTHOQUINONE AND MENAQUINONE BIOSYNTHESIS.

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Received July 22, 1970

SUMMARY.

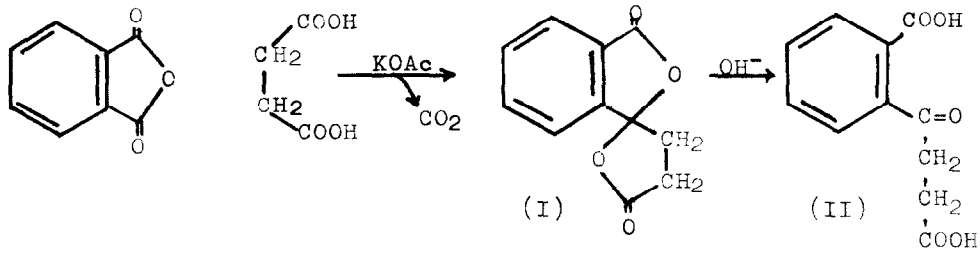
[^{14}C] o-succinylbenzoic acid (OSB) has been synthetized and found to be efficiently incorporated into bacterial menaquinones and into some plant naphthoquinones and anthraquinones. Degradation of these molecules showed that OSB is incorporated without randomisation. A tentative general scheme for naphthoquinone biosynthesis is proposed.

Shikimate has been demonstrated as an early precursor of bacterial menaquinones and of some plant naphthoquinones and anthraquinones (1, 2, 3). Some indications from bacterial mutants(especialy Aerobacter aerogenes 170-44) have been obtained suggesting that chorismate might be also an intermediate (2, 3). However Leistner and Zenk (4) failed to incorporate this compound into juglone, a quinone from Juglans regia known to derive from shikimate.

It was postulated in a preceding paper (3) that 2 of the 3 missing carbon atoms of the naphthoquinone ring could originate from the enoylpyruvic moiety of chorismate through a migration of this moiety to the C_6 atom, the third missing carbon atom coming from malonate (4). Recently, however, Campbell (5) and Robins (6) reported that the 3 missing carbons originated more or less directly from C_{2-3-4} of glutamate; the pathway suggested was transamination to 2-ketoglutarate, then oxidative decarboxylation to the succinyl semialdehyde-thiamine pyrophosphate carbanion, susceptible to attack shikimate in the 6 position.

To test directly this hypothesis, it would have been necessary to prepare such an addition product. As we felt that chorismate would have more chance to be the reacting molecule than shikimate, giving directly a completely aromatic compound, we synthetized the known o-succinylbenzoic acid II (OSB)

obtained as its dilactone I by condensation of phthalic anhydride with succinic acid (7).



When Escherichia coli mutants 83-1 and 156-53, blocked in the early reactions of aromatic biosynthesis (8) and unable to make menaquinone and ubiquinone, were fed with an aromatic supplement and 10^{-4} M OSB, they formed the same normal amount of menaquinone as when fed with 0.2×10^{-4}M shikimate. With E.coli 159-4, which is blocked after shikimate, only OSB was effective to form menaquinone; the same was observed with Aerobacter aerogenes 170-44 which is blocked after 3-enolpyruvyl shikimic 5-phosphate (2). These results establish that OSB is a possible immediate precursor for the naphthoquinone ring, and that the cross-point for this biosynthesis and general aromatic biosynthesis is at chorismate.

To obtain a clearer evidence, [^{14}C]-OSB was synthetized from [^{14}CO]-phthalic anhydride and the labeled compound administered to Mycobacterium phlei, E.coli K_{12} and A. aerogenes 62-1.

M. phlei incorporated nearly all the label into MK-9 (II-H_2) with a low dilution factor; similar low dilution factors were observed with E.coli and A.aerogenes but were associated with a much lower utilization of the radioactive compound (80-95 % of it could be recovered unchanged from the growth medium). However appreciable amounts of radioactivity were incorporated into both menaquinone-8 and demethylmenaquinone-8 carefully purified by TLC, reversed phase paper chromatography and silver nitrate impregnated TLC (9).(Table I). Radioactivity associated with ubiquinone, when present, was negligible. In all cases, it seems that menaquinone and demethylmenaquinone were the only labeled compounds detectable.

High incorporations were also observed in lawsone or juglone when

TABLE I

Incorporation of [^{14}C]-OSB into bacterial menaquinones

	[^{14}C]-OSB administered	quinone	specific activity dpm/μmole	% incorp.	dilution factor
M.phlei	2.38 x 10^6dpm[a] (2 μC/μmole)	MK-9(II H_2)	82,500	70	0.0187
A.aerogenes 62-1	3.57 x 10^6dpm[b] (2 μC/μmole)	MK-8	6,200	0.18	0.0014
		DMK-8	17,050		0.0038
		Q-8	37		
E.coli K-12	8.16 x 10^6dpm[c] (5 μC/μmole)	MK-8	880,000	2.25	0.08
		DMK-8	2,040,000		0.185
		Q-8	7,100		

[a] 0.13 x 10^6 dpm remaining in the medium, [b] 2.8 x 10^6 dpm in the medium,
[c] 7.68 x 10^6 dpm in the medium.

TABLE II

Incorporation of [^{14}C]-OSB into plant naphthoquinones

	[^{14}C]-OSB administered	quinone	specific activity dpm/μmole	% incorporation	dilution factor
Impatiens balsamina	7.5 μC[a] (2 μC/μmole)	lawsone	19,600	20	0.0045
Juglans regia	1 μC[b] (5 μC/μmole)	juglone	1,100	6	0.0001
Rubia peregrina	4 μC[c] (5 μC/μmole)	pseudo-purpurine	1,160	1	0.0001
		rubiadine	398		0.00003

[a] six 5 week's old plants during 48 h.; all the radioactive precursor absorbed;
[b] five young shoots during 60 h; 90 % absorbed;
[c] eight roots (12 g) during 50 h; 58 % absorbed.

TABLE III

Degradation of radioactive quinones.

		specific activity (dpm/μmole)	% radioactivity
M.phlei	MK-9(II-H_2)	1,500	100
	phthalic acid	1,465	97.5
	CO_2 [a]	660	44
I.balsamina	lawsone	625	100
	phthalic acid	621.5	99.5
	CO_2 [a]	290	46.5
J.regia	juglone	1,100	100
	3-hydroxyphthalic acid	1,165 [c]	106
	CO_2 [b]	562	51
	3-hydroxybenzoic acid	554	50.3
R.peregrina	pseudopurpurine	1,160	100
	phthalic acid	1,100	95

[a] CO_2 from decarboxylation of phthalic acid (3, 9).

[b] CO_2 from monodecarboxylation of 3-hydroxyphthalic acid (4)

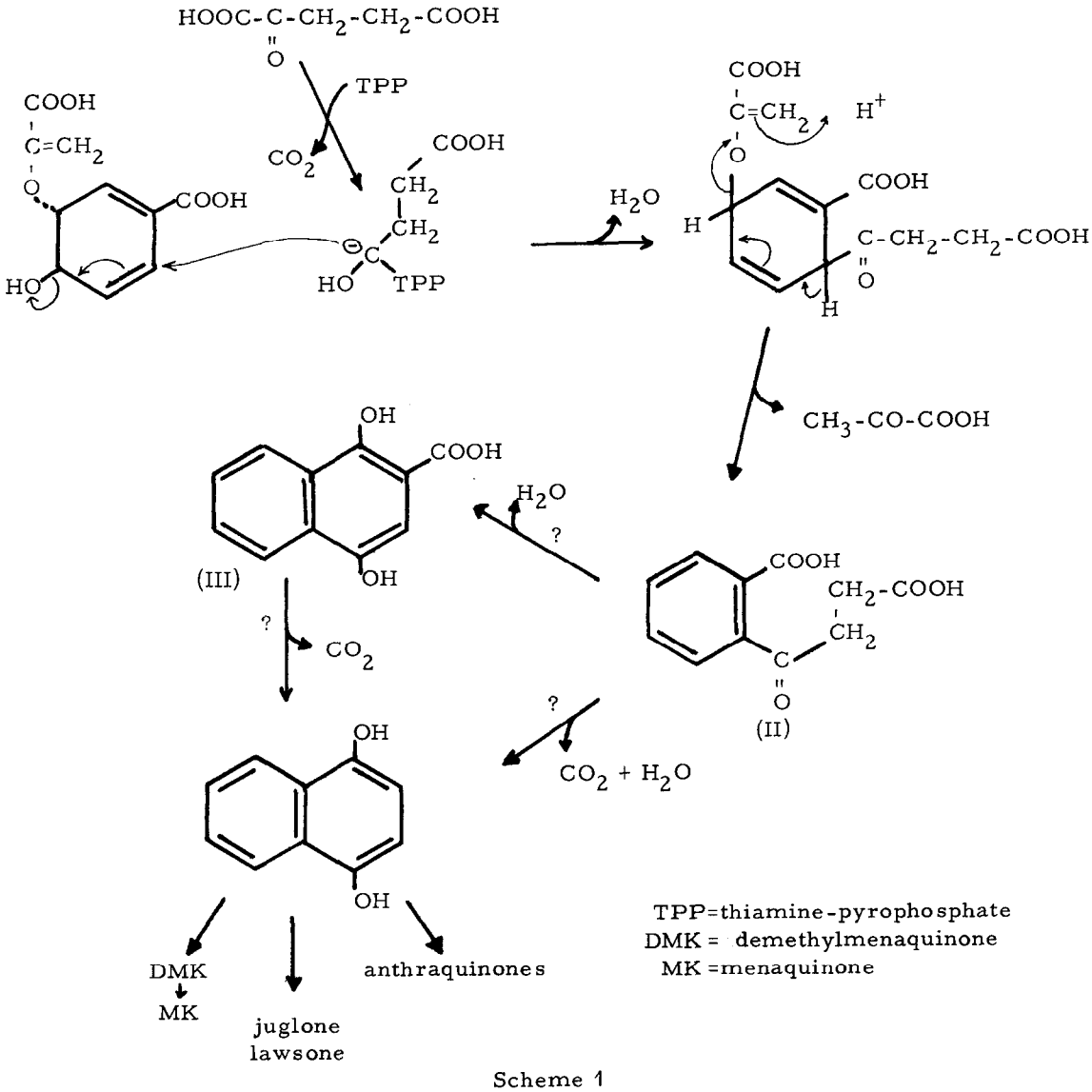
[c] specific activity of 3-hydroxyphthalic acid based on ϵ^{MeOH}_{306nm} =3520.

[^{14}C]-OSB was administered to young shoots of Impatiens balsamina or Juglans regia (Table II). Anthraquinones of Rubia peregrina (which have been demonstrated to derive from the naphthoquinone ring (10)) exhibited a similar high incorporation.

All the radioactivity of MK-9(II-H_2) was recovered in phthalic acid obtained by $KMnO_4$ oxidation; decarboxylation of this acid showed that all the label was exclusively localized in C_1 and C_4 of the naphthoquinone ring (Table III). Analogous degradation of lawsone, juglone and pseudopurpurine gave essentially identical results.

It can be deduced from these experiments that OSB is the true intermediate for the biosynthesis of its naphthoquinone ring. The corresponding dilactone is a less efficient precursor for lawsone in I.balsamina.

From these results it is clear, at least in bacteria, that OSB can only originate from the condensation, with dehydration, of chorismic acid* with a C_4 derivative which might be the succinyl-semialdehyde thiamine-pyrophosphate carbanion postulated by Campbell (5, 6). This condensation could lead through a simple elimination mechanism to the fully aromatic OSB (scheme 1).



Scheme 1

*An alternative possibility, more attractive in terms of Michael addition on a carboxyl-activated double bond, would be an attack of the same carbanion on isochorismic acid, enzymatically formed from chorismate (13) followed by dehydration. However isochorismate has been until now only associated with 2, 3-dihydroxybenzoic acid biosynthesis.

Adequate activation of this compound would permit a Claisen intramolecular condensation with/or without concomittant decarboxylation. The unstable 1,4-dihydroxy 2-naphthoic acid (III) (11) has been prepared. When this compound was fed together with [^{14}C]-OSB to I.balsamina, radioactivity incorporation into lawsone was found unchanged. However this result cannot constitute a definitive argument against 1,4-dihydroxy 2-naphthoic acid participation in the biosynthetic pathway to naphthoquinones.

The last compound of such a scheme would be naphthohydroquinone, able to react directly with a polyprenylpyrophosphate (12) to form a demethylmenaquinone.

Acknowledgments: The authors are greatly indebted to Professor E.LEDERER for helpful discussions and encouragements. This research was supported by a subvention from the Commissariat à l'Energie Atomique for radioactive compound supply.

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