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BIOSYNTHESIS OF FURANOSESQUITERPENOID STRESS METABOLITES IN *IPOMOEA BATATAS*: ISOTOPIC OXYGEN INCORPORATION INTO IPOMEAMARONE

LEO T. BURKA and ALICE THORSEN
Department of Chemistry, Vanderbilt University, Nashville, TN 37235, U.S.A.

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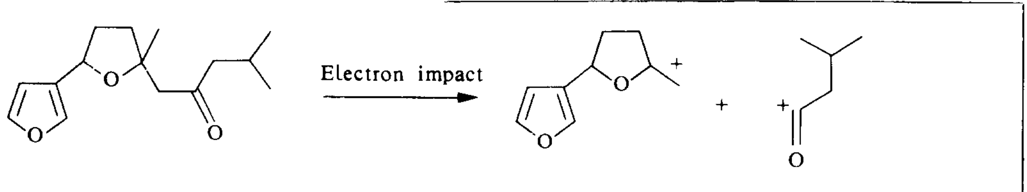
Key Word Index—*Ipomoea batatas*; Convolvulaceae; sweet potatoes; sequiterpene; biosynthesis; ipomeamarone; ^{18}O.

Abstract—All three oxygen atoms in ipomeamarone, a major stress metabolite of the sweet potato, were found to originate from molecular oxygen and not from water or the sesquiterpene precursor, farnesol.

INTRODUCTION

In recent years we have been concerned with the biosynthesis of the furanosesquiterpenoid stress metabolites of the sweet potato. The precursor–product relationship of many of these compounds has been established by us [1,2] and by Uritani's group in Japan [3–5]. As a consequence of these investigations we have been interested in the source of the oxygen atoms in these stress metabolites. If oxygen is incorporated as a result of allylic oxygenation by a cytochrome P450-containing enzyme or an enzyme with a similar oxygenating mechanism, then oxygen incorporation from O_2 would be expected, at least for side chain oxygens. The source of the furan oxygen is not obvious, as it could be the original farnesol oxygen or perhaps added later as the result of hydrolysis or oxygenation. Incorporation of $^{36}O_2$ and $H_2{}^{18}O$ into ipomeamarone (**1**) has been investigated to determine the source of the oxygen atoms in this compound.



Ipomeamarone **1**   *m/e* 151   85

RESULTS AND DISCUSSION

Sweet potato tissue was treated with 1% meruric chloride solution in the presence of either $H_2{}^{18}O$ or $^{36}O_2$. After 2–3 days ipomeamarone was isolated. Incorporation was monitored by GC/MS. Stressing sweet potato tissue in the presence of $H_2{}^{18}O$ did not result in incorporation of label into ipomeamarone.

The mass spectrum of ipomeamarone from incubation in an $^{36}O_2$ atmosphere showed peaks at *m/z* 250, 252, 254 and 256 resulting from molecules containing zero, one, two and three ^{18}O atoms respectively. The ratio of the intensity of peaks was 32:43:21:4. Calculation of the expected ratio from a 71:29 ratio of ^{16}O:^{18}O gave 37:44:17:2[6]. The major fragmentation process for ipomeamarone gives an ion at *m/z* 151. For the ^{18}O-containing compound isotopic peaks were observed at *m/z* 151, 153 and 155 in a ratio of 45:44:12. The calculated ratio is 51:41:8. Finally, a major fragment normally at *m/z* 85 gave an isotopic peak at *m/z* 87 (90:10 ratio). The expected ratio is 71:29. Only this last ion ratio varies significantly from the calculated ratio. This may result from the presence of ions with *m/z* 85 that contain no oxygen. We have no high resolution data to clarify this point.

From these results it appears that all the oxygen atoms in ipomeamarone are derived from O_2. The original farnesol oxygen is not found in the product; thus, any mechanism for furan ring formation must involve oxygen from O_2 and loss of the farnesol hydroxyl (probably as pyrophosphate). A possible mechanism involves oxidation of the proximate methyl to an alcohol or aldehyde followed by displacement of pyrophosphate and subsequent oxidation and dehydration (or simply dehydration in the case of prior oxidation to aldehyde). The intermediate dihydrofurans are not known but should be synthetically accessible for feeding studies.

EXPERIMENTAL

Ipomeamarone was isolated by homogenizing the sweet potato tissue in MeOH–CH_2Cl_2(1:2) with addition of H_2O as necessary to form two layers. After removal of the CH_2Cl_2 the residue was subjected to TLC on Si gel using EtOAc–hexane (1:9) as the solvent.

GC/MS analysis was performed on a Ribermag model

R10-10. A 6 ft 3% OV-17 column programmed from 80° at 8°/min was used for ipomeamarone. A 6 ft 13X molecular sieve column at room temp. was used for O_2 analysis. The isotopic ratios were derived from at least three spectra from the GC peak.

$H_2{}^{18}O$ *incorporation.* Sweet potato slices (10 g) were placed in a Petri dish with 0.5 ml $H_2{}^{18}O$ (95–99% ^{18}O) containing 1% $HgCl_2$ and the dish sealed with tape. The dry wt–wet wt ratio of similar slices was found to be 0.20. If it is assumed that all the volatile material is H_2O then the slices should contain 6% $H_2{}^{18}O$ and 94% $H_2{}^{16}O$. After 3 days the ipomeamarone was isolated and analysed by GC/MS. The ratio of m/z 250 and 252 peaks was identical to natural abundance.

$^{36}O_2$ *incorporation.* Slivers of sweet potato (*ca* 3 × 3 × 30 mm, 5 g) treated with 1% $HgCl_2$ were placed in a Thunberg tube. The tube was evacuated with a water aspirator and refilled with a mixture of N_2 and O_2 (2:1). The atmosphere in the tube was immediately analysed; the O_2 was 71% $^{32}O_2$, 28.5% $^{36}O_2$ and 0.5% $^{34}O_2$. After 3 days at room temp. the ipomeamarone was isolated and subjected to GC/MS analysis.

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