Phytochemistry, 1977, Vol. 16, pp. 2022–2023. Pergamon Press. Printed in England.

BIOSYNTHESIS OF FURANOSESQUITERPENOID STRESS METABOLITES IN SWEET POTATOES (*IPOMOEA BATATAS*). OXIDATION OF IPOMEAMARONE TO 4-HYDROXYMYOPORONE

LEO T. BURKA and LEE KUHNERT

Department of Chemistry, Vanderbilt University, Nashville, TE37235, U.S.A.

(*Received* 30 *April* 1977)

Key Word Index—*Ipomoea batatas*; Convolvulaceae; sweet potatoes; sesquiterpene; biosynthesis; pomeamarone; 4-hydroxymyoporone.

INTRODUCTION

In the forty years since the isolation of ipomeamarone, (**1**), from sweet potatoes a vast amount of work has been carried out on the furanosesquiterpenoid stress metabolites of this plant [1]. In that time 10–15 furans have been isolated from this source which have unrearranged sesquiterpene skeletons; some, such as batatic acid and 4-ipomeanol, are apparently degraded sesquiterpenes. While most of our attention has been focused on 4-ipomeanol and the other lung toxins, we have beeen interested in the oxygenation steps which must take place in the plant to transform farnesol to the stress metabolites. This report concerns the conversion of ipomeamarone (**1**) to 4-hydroxymyoporone (**2**). Compound **2** has been shown to be metabolized to the lung toxins by the sweet potato pathogen, *Fusarium solani* [2].

RESULTS AND DISCUSSION

The results from experiments 1 and 2 in Table 1 show that ipomeamarone (**1**) is efficiently incorporated into 4-hydroxymyoporone (**2**). Over 1% of the administered radiolabel was recovered in **2**. The results also indicate that ipomeamarone (**1**) was being produced by the plant during the experiment. No **1** was present in the plant prior to pretreatment with mercuric chloride but the specific activity of **1** recovered after the experiment was about 50% that of the proferred material. Thus, it appears that sesquiterpene biosynthesis is not greatly perturbed by addition of **1**.

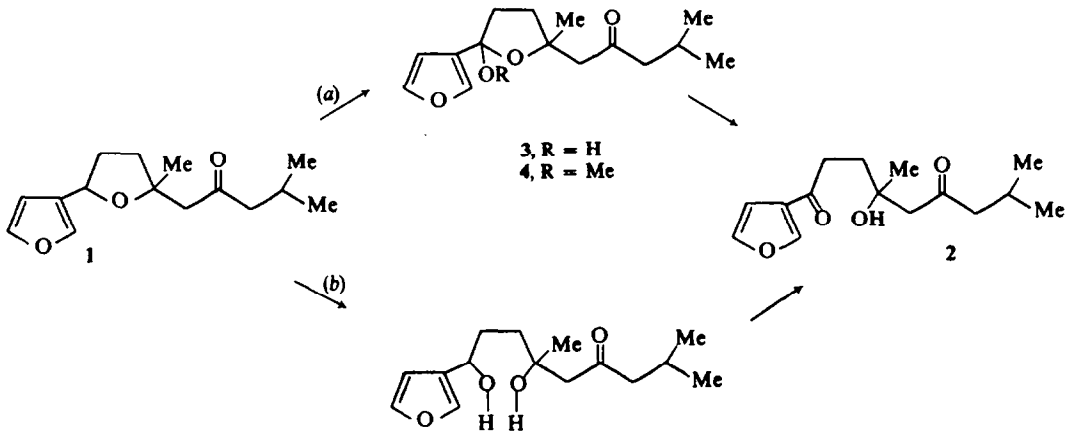
If **1** is an intermediate on the biosynthetic pathway to **2**, addition of unlabelled **1** along with acetate-[2-^{14}C] should result in decreased incorporation of radioactivity into **2**. In concurrent experiments (3 and 4) acetate-[2-^{14}C] (60 μCi, 1 μmol) was added to mercuric chloride-stimulated sweet potato slices. Unlabelled **1** was added along with the acetate in experiment 3; in experiment 4 no **1** was added. Addition of unlabelled **1** resulted in a 3-fold decrease in the incorporation of acetate and an increase in the dilution of label in the **2** which was formed. These results are consistent with the hypothesis that ipomeamarone (**1**) is a precursor of **2**. The efficient incorporation, and especially the low dilution values, argue against incorporation resulting from fragmentation of **1** followed by synthsis of **2** from some of the fragments.

Two plausible pathways for the oxidation of ipomeamarone to **2** are shown in Scheme 1. Oxygenation to form hemiketal **3** (path *a*) seems most reasonable in view of recent reports of oxygenation at allylic positions of terpenes in other plants [3,4]. In fact, path *a* can be mimicked by treating **1** with cuprous chloride and *t*-butyl perbenzoate in methanol. This reagent is known to oxidize ethers to ketals [5]. 4-Hydroxymyoporone produced by hydrolysis of ketal **4** has the same sign and magnitude of optical rotation as samples of this metabolite which have been isolated from sweet potatoes. Other pathways, such as hydrolysis of the tetrahydrofuran ring followed by oxidation (path *b*) cannot be ruled out.

Table 1. Incorporation of ^{14}C-precursors into 4-hydroxymyoporone

Experiment	Precursor		Product		% Incorporation into **2**
	Ipomeamarone-[^{14}C]	Acetate-[2-^{14}C]	Ipomeamarone (**1**)	4-Hydroxymyoporone (**2**)	
	Specific activity, 10^7 dpm/mmol				
1	1.77	—*	0.92(2.0)†	0.40(4.5)	1.4
2	1.77	—*	0.72(2.5)	0.52(3.5)	1.1
3	—‡	13 000	—	0.76	0.09
4	—§	13 000	—	1.83	0.29

*No acetate-[2-^{14}C] added.
†Number in parenthesis is the dilution value.
‡Unlabelled ipomeamarone (0.3 mg/g of tissue) added.
§No ipomeamarone added.



Scheme 1.

EXPERIMENTAL

Ipomeamarone-[^{14}C] (1.77×10^7 dpm/mmol) was prepared by treating sweet potato slices with $HgCl_2$ in the presence of acetate-[2-^{14}C] and isolated via the semicarbazone derivative [2]. Sweet potatoes (surface sterilized with sodium hypochlorite soln) were cut into 75 mm thick slices, dipped into 15% mannitol soln and placed in sterile petri dishes. A 1% $HgCl_2$ soln and a suspension of ipomeamarone-[^{14}C] in Tween-80 and H_2O was distributed on the surface of the slices. $HgCl_2$ was used to induce stress metabolite production since its effect is almost immediate with no lag-time for fungus growth. The use of $HgCl_2$ also eliminates the possibility of further metabolism of the stress metabolites by the fungus [2]. The concn of ipomeamarone used, 0.3 mg of **1** per gram of tissue is *ca* the concn found in mould-damaged sweet potatoes [6]. After 5 days the furans were isolated by extraction with MeOH followed by PLC on 1 mm Si gel plates developed twice with EtOAc–hexane (1:3).

The sp. act. of recovered (**1**) was determined by derivatization as the semicarbazone and recrystallization to constant activity. The amount of 4-hydroxymyoporone [^{14}C] isolated was determined by HPLC, the *uv* detector response to **2** was linear over the range used. The 4-hydroxymyoporone-[^{14}C](**2**) was then diluted about $\times 10$ with a known weight of unlabelled **2**. No suitable solid derivative of **2** has been found, and in order to demonstrate radiochemical purity the diluted material was subjected to HPLC on a 120 cm (about 12000 theoretical plates) corresponding to **2** was then recycled on the column and μ-Porasil column. The peak corresponding to **2** was then recycled on the column and collected as 2 or 3 fractions. The standard error of the 2–3 determinations of sp. act. for each run was 5% or less. Since the sp. act. remained constant through the peak we take this as an indication of radiochemical purity. The sp. act. of **2** reported in Table 1 is the calculated sp. act. of the isolated 4-hydroxymyoporone-[^{14}C]-(**2**) before addition of unlabelled **2**.

Acknowledgements—This work was supported by Center in Toxicology Grant ES00267 to Vanderbilt University from the U.S. Public Health Service. The authors wish to thank Prof. T. M. Harris for helpful discussions.

REFERENCES

1. Burka, L. T. and Wilson, B. J. (1976) in *Mycotoxins and Other Related Food Problems* (Rodricks, J. V. ed.) pp. 387–399. American Chemical Society, Washington.
2. Burka, L. T., Kuhnert, L., Wilson, B. J. and Harris, T. M. (1977) *J. Am. Chem. Soc.* **99**, 2302.
3. Fujita, T., Masuda, I., Takao, S. and Fujita, F. (1976) *J. Chem. Soc. Perkin.* **1**, 2098. (1976)
4. Madyastha, K. M., Meehan, T. D. and Coscia, C. J. (1976) *Biochemistry* **15**, 1097.
5. Lawesson, S. -O. and Berglund, C. (1961) *Angew. Chem.* **73**, 65.
6. Boyd, M. R. and Wilson, B. J. (1971) *J. Agr. Food Chem.* **19**, 547.