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SHORT COMMUNICATION

INCORPORATION OF MEVALONIC ACID-2-^{14}C INTO THE TRITERPENE LIMONIN

SIBANI DATTA and HAROLD J. NICHOLAS

Institute of Medical Education and Research, and the Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri, U.S.A.

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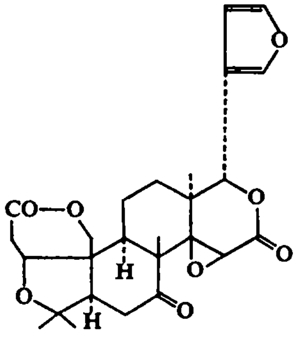
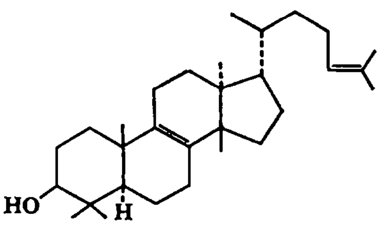
Abstract—All portions of two kinds of orange plants (*Citrus* species) were tested for the ability to incorporate mevalonic acid-2-^{14}C into the triterpene limonin. Only the seeds were able to form radioactive limonin. A method is described for preparing limonin-^{14}C.

INTRODUCTION

IT HAS been proposed[1] that the precursor of the triterpene limonin (I) is a tetracyclic triterpene of the euphol (II) type, but experimental evidence is lacking. This and other problems involved in the biosynthesis of limonin can be approached by stepwise chemical degradation of limonin-^{14}C. The object of the present study was to ascertain the best method of preparing the labeled triterpene for degradation. Succeeding manuscripts will discuss the chemical degradation and the specific nature of the precursor(s) of limonin. Both the Calamondin orange and the Valencia orange were investigated for this purpose. Only the seeds[2, 3] of these species contained limonin, and the synthetic capacity for the triterpene was found to reside in this tissue.



I limonin



II euphol

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[1] D. ARIGONI, D. H. R. BARTON, E. J. COREY, O. JEGER, L. CAGLIOTA, SUKH DEV, P. G. FERRINI, E. R. GLAZIER, A. MELERA, S. K. PRADHAN, K. SCHAFFNER, S. STERNHELL, J. F. TEMPLETON and S. TOBINAGA, *Experientia* 16, 41 (1960).

[2] DAVID L. DREYER, *Phytochem.* 5, 367 (1966).

[3] OLIVER H. EMERSON, *J. Am. Chem. Soc.* 70, 545 (1948).

RESULTS AND DISCUSSION

A number of methods were investigated in efforts to obtain limonin-^{14}C from mevalonic acid-2-^{14}C, including injecting mevalonic acid solution directly into orange fruits or absorption of the solution from a small vessel by cut stems containing orange fruits. Both of these methods effectively labeled β-sitosterol and citrostadienol under these conditions in all portions of the fruit, stems and leaves,[4] but no radioactive limonin was obtained except from the seeds. However, limonin from the latter was too weakly radioactive even after massive injection of mevalonic acid. The only effective method of obtaining limonin-^{14}C was to feed precursor directly to the seeds as described in the Experimental section. Co-crystallization to constant radioactivity of two limonin-^{14}C preparations with authentic non-radioactive limonin is shown in Table 1.

TABLE 1. CO-CRYSTALLIZATION OF LIMONIN-^{14}C WITH NON-RADIOACTIVE LIMONIN

	(mg)	Total (counts/min)	Sp. act. (counts/min/mg)
Preparation*			
Initial mixture‡	49·9	19,860	398
1st Crystallization	31·2	13,120	420
2nd Crystallization	23·4	9,620	410
3rd Crystallization	19·0	7,960	418
Preparation 2†			
Initial mixture§	24·3	36,520	1,503
1st Crystallization	21·9	31,420	1,433
2nd Crystallization	18·4	26,650	1,398
3rd Crystallization	14·1	19,430	1,381

* Following incubation with mevalonic acid-2-^{14}C for 2 days.
† Following incubation with mevalonic acid-2-^{14}C for 6 days.
‡ 46 mg authentic limonin plus 3·9 mg limonin-^{14}C.
§ 22·3 mg authentic limonin plus 2·0 mg limonin-^{14}C.

Although the percent incorporation of mevalonic acid-2-^{14}C into limonin was small, the method is capable of supplying limonin-^{14}C for chemical degradation. It is of interest that the Navel orange produces no seeds but the juice from this species develops limonin on standing.[3]

EXPERIMENTAL

Mevalonic acid-2-^{14}C as the dibenzylethylenediamine salt (100 μc; 8·2 mg) in 0·8 ml H_2O was distributed uniformly over sixty-five seeds (11·07 g) of germinated Valencia orange. After complete absorption of the solution (about 2 hr) the seeds were kept moist for different periods, then washed thoroughly in distilled H_2O and dried. They were then finely ground and extracted successively with light petroleum (b.p. 30–60°), benzene and ethanol. The benzene extracts containing the limonin (about 200 mg) were chromatographed on activated silica gel (Mallinckrodt, 200 mesh, 15 g, 1 cm dia. column). Fractions (40 ml) were collected as follows: 1 to 9, benzene; 10 to 14, benzene–acetone (19:1); 15 to 20, benzene–acetone (9:1); 21 to 38, benzene–acetone (4:1). Fraction 25 contained limonin; 50 μg gave a single spot ($R_f = 0·34$) when subjected to TLC on 0·25 mm silica gel G plates in benzene–acetone (4:1), and the triterpene gave a light-green color with 20 per cent $SbCl_5$ in $CHCl_3$. In the same solvent system the following "limonoids" gave R_f's as indicated: Obacunone, 0·56; Nomilin, 0·40; Deacetylnomilin, 0·20.

The incorporation of mevalonic acid-2-^{14}C into limonin was 0·03 and 0·05% after incubation of the seeds for 2 and 6 days respectively.

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[4] Unpublished observations.