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SHORT COMMUNICATION

BIOSYNTHESIS OF URSOLIC ACID-2-^{14}C

H. J. Nicholas

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

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Abstract—Ursolic acid-^{14}C was prepared by administration of mevalonic acid-2-^{14}C, sodium acetate-1- and -2-^{14}C, sodium formate-^{14}C and L-methionine-methyl-^{14}C respectively to cut sections of *Salvia officinalis*. Only mevalonic acid-2-^{14}C was incorporated efficiently into the acidic triterpene. Each of the ursolic acid preparations was subjected to the Schmidt reaction. The C-28 of ursolic acid acquired a small amount of label from formate-^{14}C, but not from any other of the precursors. The reason for this is not known.

INTRODUCTION

As an approach to the chemical degradation of a triterpene labeled biosynthetically with ^{14}C, ursolic acid-^{14}C was prepared from several labeled precursors. This was performed by administering the precursors to *S. officinalis* cut stems since the plant is a convenient source of ursolic acid. The labeled ursolic acids were then subjected to the Schmidt reaction,[1] which has been used successfully in ascertaining that the methyl group of acetic acid is the source of the carboxyl group in eburicoic acid.[2]

RESULTS AND DISCUSSION

The per cent incorporation of ^{14}C from the various labeled precursors into ursolic acid-^{14}C is shown in Table 1. Except for mevalonic acid-2-^{14}C, the precursors were uniformly incorporated rather inefficiently by this method.

Table 1. Incorporation of ^{14}C-labelled precursors into ursolic acid of *S. officinalis*

^{14}C-Precursor	Amount (μg)	Wet weight of plant (g)	Ursolic acid ^{14}C yield (mg)	Total ^{14}C incorporation, c.p.m. $\times 10^{-4}$	Per cent incorporation of radioactivity
Mevalonic acid-2-^{14}C	20	158	679	100·0	21·02
Sodium acetate-1-^{14}C	400	842	6210	23·6	0·22
Sodium acetate-2-^{14}C	400	878	4500	6·5	0·06
Sodium formate-^{14}C	400	314	760	2·6	0·02
Sodium formate-^{14}C	2000	87	20*	6·2	0·01
L-Methionine-methyl-^{14}C	100	61	28*	5·5	0·21

* Diluted with 100 mg of non-radioactive ursolic acid prior to further purification.

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[1] H. Wolff, In *Organic Reactions*, Vol. 3, p. 307. John Wiley, New York (1946).

[2] W. G. Dauben, Y. Ban and J. H. Richards, *J. Am. Chem. Soc.* **79**, 968 (1957).

When each of the ursolic acid-^{14}C preparations were subjected to the Schmidt reaction the results shown in Table 2 were obtained. It will be noted that only ursolic acid-^{14}C prepared from sodium formate-^{14}C gave rise to radioactive carbon dioxide. Of the precursors utilized, only sodium acetate-2-^{14}C should label the C-28 methyl group of the ursane nucleus, or the carboxyl resulting from biological oxidation of this group. The observation, therefore, has no obvious explanation at the present and will bear further investigation.

TABLE 2. RECOVERY OF $^{14}CO_2$ FROM URSOLIC ACID-^{14}C PREPARED BIOSYNTHETICALLY FROM VARIOUS ^{14}C-PRECURSORS AND SUBJECTED TO THE SCHMIDT REACTION

	Ursolic acid-^{14}C subjected to reaction			$BaCO_3$		Total ^{14}C in $BaCO_3$		
^{14}C-Precursor	Sp. act. c.p.m./mg C	(mg)	Total c.p.m. × 10^{-3}	Found mg	Per cent recovery	Found c.p.m.	Calc. c.p.m.	Yield %
Mevalonic acid-2-^{14}C	2160	500	849	208	95	0	—	0
Sodium acetate-1-^{14}C	1285	500	505	219	100	0	—	0
Sodium acetate-2-^{14}C	682	500	268	141	65	0	—	0
Sodium formate-^{14}C	107	355	30	66	44	260	1000	26*
Sodium formate-^{14}C	296	82	19	40	92	620	633	98
L-Methionine-methyl-C^{14}	474	59	22	25	100	0	—	0

* 59% if corrected to 100% recovery of $BaCO_3$.

EXPERIMENTAL

The method of administering the ^{14}C-labeled precursors has been previously described.[3-5] Sodium formate-^{14}C was obtained from Volk Radiochemicals, Skokie, Illinois. All other labeled precursors were purchased from the New England Nuclear Corp., Boston, Massachusetts. After absorption of the ^{14}C-precursor solutions the plant sections were kept in distilled H_2O for a week, then allowed to dry an additional week. The plant tissue was then extracted exhaustively with ethanol and the extracts were distilled to dryness. The residues were saponified, "crude acidic triterpene" fractions were isolated and chromatographed on deactivated alumina as described previously.[3] Ursolic acid was isolated from the 5% ethanol (in benzene) eluates, acetylated and crystallized to a constant m.p. of 283–286°. Saponification and several crystallizations from aqueous ethanol gave ursolic acid, m.p. 285–287°. The Schmidt reaction was then performed as described by Dauben and Richards.[2] The evolved CO_2 was collected as $BaCO_3$. Samples to be counted for ^{14}C were plated as infinitely thin layers and counted in a windowless gas-flow counter. Barium carbonate samples were corrected to infinitely thick layers by means of a standard $Ba^{14}CO_3$ curve prepared with the particular instrument used.

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[3] H. J. NICHOLAS, *J. Biol. Chem.* **237**, 1476 (1962).
[4] H. J. NICHOLAS, *J. Biol. Chem.* **237**, 1481 (1962).
[5] H. J. NICHOLAS, *J. Biol. Chem.* **237**, 1485 (1962).