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ON THE BIOSYNTHESIS OF THE TERPENE MARRUBIIN FROM [1,4-^{14}C]SUCCINIC ACID AND [2-^{14}C] MEVALOLACTONE.

Ada Abbondanza[a], Roberto Badiello[b] and Alberto Breccia
Centro Nazionale di Chimica delle Radiazioni e dei Radioelementi del C.N.R. Sezione III. Università di Bologna, Italia.

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The fundamental isoprene rule in the biogenesis of terpenes namely Ruzicka's theory (1), includes the condensation of acetate molecules to mevalonic acid, transformation of the latter to isopentenyl pyrophosphate and condensation of these isoprenic units to terpenes.

In the preliminary study on the pathways leading to the formation of marrubiin, Ruzicka's theory was applied by administration of ^{14}C labelled acetate and mevalolactone to flowering plants of Marrubium vulgare L. Further it was considered of interest to investigate the possible participation of succinic acid in the biosynthesis of the terpene in accordance with the biogenesis of other natural substances (2,3,4).

The flowering Marrubium vulgare was cut at the base of the stem at root level and 500 gr of fresh tissue was grown in 50 ml radioactive solution and 50 ml distilled water added every 10 hours. After a set period of metabolism, the marrubiin was extracted from the dried plants in accordance with previous work (3).

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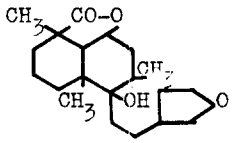
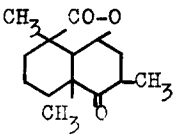
[a]Fellow of the C.N.R. Italy.

[b]Istituto Chimico "G.Ciamician" Università di Bologna. ITALIA

The degradation of marrubiin to the corresponding ketolactone in order to determine the distribution of radioactivity between the naphthalenic and furanic moieties, was made according to the published technique (5).



marrubiin



ketolactone

The radiochemical measurements were made with a scintillation counter supplied by S.E.L.C. for liquid phase scintillators. 2,5-Diphenyloxazole (PPO) as primary scintillator and 1,4-bis--2-(5-phenyloxazolyl)-benzene (POPOP) as secondary scintillator were used in toluene solution.

The efficency of the instrument was determined by measuring standard samples of [^{3}H,^{14}C] n-hexadecane using identical experimental conditions. The radioactivity data are reported to the 100% efficency. The error for the standard deviation is 3%. The radiochemical purity was confirmed by isotope dilution analysis, using stable marrubiin.

Table I shows the specific activity in μC/mM and the specific incorporation, at different times, in samples of marrubiin obtained from [1,4-^{14}C] succinic acid.

Table III shows the value of the radioactivity, obtained from the degradation of marrubiin.

The effective incorporation of used precursors is evident from the data and the rule of acetate condensation to mevalolactone is confirmed according to Tchen's theory (6) (Table II).

In this preliminary comunication the kinetics of incorporation of [1,4-^{14}C] succinic acid in marrubiin (Table I) is correlated with the kinetics of other precursors in a further evaluation of the intermediate steps in marrubiin biogenesis.

Degradation of the marrubiin labelled from [2-^{14}C] mevalolactone and from [1,4-^{14}C] succinic acid, yields a ketolactone with a specific activity (d.p.m./mM) of 70% and 68% respectively referred to the terpene.

On the basis of a molecular structure regarding marrubiin as a diterpene, it must contain four isoprenic units.

The radioactivity data on the incorporation of [1,4-^{14}C] succinic acid and [2,3-^{3}H] succinic acid (Table II), suggest that at least three carbon atoms of each succinic acid unit are involved in the marrubiin biogenesis. In fact, both labelled compounds are incorporated.

Moreover, it is suggested that the pathway of incorporation of [1,4-^{14}C] succinic acid into marrubiin via isoprenic units could involve only one -^{14}COOH of the acid for each isoprenic unit. In such a way we could have four ^{14}C in the marrubiin and three ^{14}C in the ketolactone, a 75% of the total incorporated radioactivity. In table III it is shown that the remaining activity in the ketolactone is 68% in good agreement with the above suggested data.

Also in the degradation product of marrubiin from [2-^{14}C] mevalolactone, three ^{14}C carbon atoms should remain with 75% of the total radioactivity in agreement with the found radioactivity as shown in Table III.

Other experiments on the kinetics of incorporation of [2-^{14}C] - mevalolactone, [1-^{14}C] acetic acid, [2-^{14}C] acetic acid, [1-^{14}C] pyruvic acid, [1,4-^{14}C] succinic acid, [2,3-^{14}C] succinic acid, [1,4-^{14}C] fumaric acid, [5-^{14}C] ketoglutaric acid and [2,5-^{14}C] citric acid as fundamental components of the "Krebs cycle" are in progress.

Table I

Precursor	Metabolism time	Spec.act. marrubiin μC/mM	Spec.act. precursor μC/mM	Specific incorporation.(*)
[1,4-^{14}C] succinic acid	12 hr	$3,00.10^{-3}$	$0,58.10^{2}$	$0,51.10^{-4}$
" "	24 hr	$3,00.10^{-3}$	"	$0,51.10^{-4}$
" "	36 hr	$7,50.10^{-3}$	"	$1,30.10^{-4}$
" "	48 hr	$10,90.10^{-3}$	"	$1,90.10^{-4}$
" "	72 hr	$17,10.10^{-3}$	"	$3,00.10^{-4}$
" "	96 hr	$27,10.10^{-3}$	"	$4,60.10^{-4}$

(*) The specific incorporation is defined as the ratio between the specific activity of the samples and the specific activity of the precursors.

Table II

Precursor	Metabolism time	Spec.act. marrubiin μC/mM	Spec.act. precursor μC/mM	Specific incorporation.(*)
[2-^{3}H] acetate	72 hr	0,13	$1,00.10^{3}$	$1,30.10^{-4}$
[1-^{14}C] acetate	72 hr	$1,70.10^{-2}$	--	--
[2-^{14}C] mevalolactone	48 hr	$3,80.10^{-2}$	$0,60.10^{2}$	$6,33.10^{-}$
[2-^{14}C] mevalolactone	72 hr	$0,70.10^{-2}$	$0,60.10^{2}$	$1,17.10^{-4}$
[2,3-^{3}H] succinic acid	72 hr	0,60	$5,00.10^{3}$	$1,20.10^{-4}$

(*) The specific incorporation is defined as the ratio between the specific activity of the samples and the specific activity of the precursors.

Table III

Precursor	Spec.act. marrubiin μC/mM	Spec.act. ketolactone μC/mM	Remaining radioactivity found	calculated
[1,4-^{14}C] succinic acid	2,20.10^{-3}	1,50.10^{-3}	68 %	75 %
[2-^{14}C] mevalolactone	3,80.10^{-2}	2,65.10^{-2}	70 %	75 %

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