Phytochemistry, 1969, Vol. 8, pp. 1705 to 1708. Pergamon Press. Printed in England

THE INCORPORATION OF MEVALONIC ACID INTO ROSE PETAL MONOTERPENES

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(*Received* 14 *January* 1969, *in revised form* 7 *March* 1969)

Abstract—Label from (±)-mevalonate-2-^{14}C has been shown to be incorporated in 10·8 per cent yield into the β-D-glucosides of the monoterpene alcohols, geraniol, nerol and citronellol by petals of the Hybrid Tea rose "Lady Seton". The results on the percentage incorporation at different times demonstrated that the monoterpene-β-D-glucosides were in a state of rapid metabolic flux.

INTRODUCTION

THE MONOTERPENES found in plant essential oils are assumed to be derived, like other isoprenoid compounds, from mevalonic acid[1–3]. As Battaile *et al.*[4] have pointed out, the experimental evidence supporting this assumption is slight, since incorporation from (±)-mevalonate-2-^{14}C into monoterpenes by whole plants or plant tissues is generally low. Although cell-free systems have been obtained which incorporated label from (±)-mevalonate-2-^{14}C into the higher terpenes in high yield,[5,6] no cell-free systems have yet produced monoterpenes in comparable yields from this precursor.

The present communication describes the incorporation, in high overall yield, of label from (±)-mevalonate-2-^{14}C into monoterpenes by the petals of the rose "Lady Seton". Our results provide supporting evidence that the *in vivo* biosynthesis of monoterpenes occurs by a mevalonic acid pathway.

RESULTS AND DISCUSSION

The flowers of the rose "Lady Seton" contain geraniol and other monoterpenes not only in the free form but also as their β-D-glucosides.[7] Since the concentrations of free and of β-D-glucoside monoterpenes increase rapidly during opening of the flowers, this stage was chosen for examination (see Experimental). Preliminary experiments were carried out with sodium bicarbonate-^{14}C and with sodium acetate-2-^{14}C. Less than 0·01 per cent of the label from sodium bicarbonate-^{14}C was incorporated into the monoterpenes, present either free or as the β-D-glucosides. By contrast, label from sodium acetate-2-^{14}C was incorporated into

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both free monoterpenes and the monoterpene components of the β-D-glucosides. Maximum incorporations of some 1 per cent were found in the β-D-glucosides 45–60 min after introduction of the label; the incorporations had decreased to low levels after 3 hr. The rate of label incorporation from (±)-mevalonate-2-^{14}C into monoterpenes was therefore followed from 15 min, the shortest practical incubation period, to 3 hr.

The maximum incorporation of label from (±)-mevalonate-2-^{14}C into the monoterpene

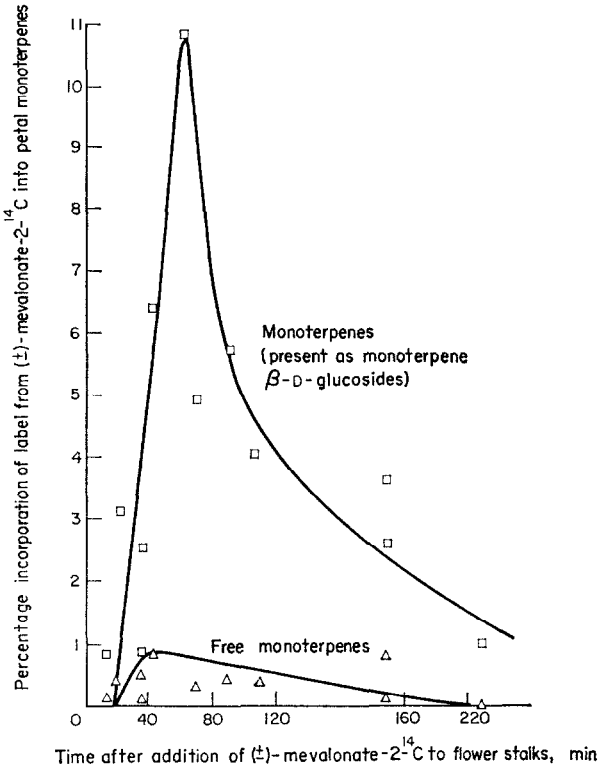

FIG. 1. INCORPORATION OF LABEL FROM (±)-MEVALONATE-2-^{14}C INTO FREE MONOTERPENES AND MONOTERPENE β-D-GLUCOSIDES BY ROSE PETALS.

Rose flowers were harvested just before the petals had fully opened out. Dibenzylethylenediamine (±)-mevalonate-2-^{14}C (900 mμmoles) was introduced via the transpiration stream by application to the cut ends of the flower stalks. Incubations were carried out at 22° and stopped by placing the flowers on dry ice. The radioactivities of the free monoterpenes and of the monoterpene β-D-glucosides isolated from the petals were estimated by radio-GLC.

moieties of the β-D-glucosides was 10·8 per cent (Fig. 1). At all times at least 88 per cent of label in the β-D-glucoside pool was accounted for by geraniol β-D-glucoside. Thus at 60 min, 10·5 per cent of the label of (±)-mevalonate-2-^{14}C had been converted to geraniol β-D-glucoside. As Fig. 1 shows, this maximum incorporation occurred roughly 60 min after application of precursor to the cut flower stalks. This rapid incorporation of label into monoterpene β-D-glucosides is followed by an equally rapid loss, demonstrating that there is a rapid flux of the carbon of (±)-mevalonate-2-^{14}C through the monoterpene β-D-glucoside pool.

All earlier reports on the incorporation of (±)-mevalonate-2-^{14}C into monoterpenes dealt exclusively with the free monoterpene pool.[1,8–11] Our results based on the monoterpene β-D-glucosides show much higher incorporations. Indeed if only (–)-mevalonate-2-^{14}C is being utilized by the petal cells[12] then the conversion into geraniol alone was 21 per cent. The levels of incorporation into the free monoterpenes were below 1 per cent, similar to those found by other workers.[1,8–11] Incorporation into sesquiterpenes (~0·1 per cent) and into higher terpenoids was also observed but was not studied further.

The rapid appearance of label in the monoterpene β-D-glucosides can be compared with the results of Battaile and Loomis[13] and of Hefendehl *et al.*[14] These workers showed that, in *Mentha piperita*, label from $^{14}CO_2$ appeared in monoterpenes within 5 min of the initial exposure to $^{14}CO_2$. Rapid interconversions between monoterpenes and higher terpenoids have also been demonstrated.[1,14] It therefore seems evident that monoterpenes are not the metabolically inert secondary products they have often been assumed to be. The present results also confirm the studies of Paseshnichenko and Guseva on isolated rose petals, which were shown to incorporate label from acetate-2-^{14}C and (±)-mevalonate-2-^{14}C into monoterpenes.[15]

The biosynthetic relationships between individual monoterpenes present free and as the β-D-glucosides were also studied. No clear pattern emerged from the kinetics of incorporation of label from (±)-mevalonate-2-^{14}C into geraniol, nerol, geraniol β-D-glucoside and nerol β-D-glucoside. One explanation is that the method of application means that (±)-mevalonate-2-^{14}C would be reaching biosynthetic sites over an appreciable proportion of the incubation time, since the precursor is taken up by cut flower stalks over a period of 8–16 min.

Previous workers have found low levels (0·01–0·1 per cent) of incorporation of label from (±)-mevalonate-2-^{14}C into monoterpenes by whole plants or by plant tissues.[1,8–11] These results have been explained in terms of the low permeability of externally applied (±)-mevalonate-2-^{14}C to the sites of biosynthesis.[1,16] The label incorporated into monoterpenes may also be derived from (±)-mevalonate-2-^{14}C indirectly. Thus in *M. piperita* (±)-mevalonate-2-^{14}C has been shown to give rise to $^{14}CO_2$ in 0·38–4·2 per cent yield over 2–14 days,[17] and $^{14}CO_2$ to yield labelled monoterpenes in up to 0·4 per cent yield under circumstances where no label from (±)-mevalonate-2-^{14}C was incorporated.[1,13] Our results could be interpreted similarly. However, low incorporations (0·01 per cent) into monoterpenes were found when sodium bicarbonate-^{14}C was used as the labelled precursor. Furthermore, petals at the partly opened stage of maturity have been shown to contain few plastids[18] and are therefore essentially non-photosynthetic tissues. These facts taken together with the high levels of incorporation and the rapid appearance of label from (±)-mevalonate-2-^{14}C in the monoterpenes and with its complete absence in β-phenylethanol, a major constituent of rose

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petals,[19] make such an explanation improbable. We conclude therefore that (±)-mevalonate-2-^{14}C has been incorporated directly into monoterpenes by rose petals.

EXPERIMENTAL

Plant Material

Plants of the rose "Lady Seton" were grown as described previously.[7] Flowers were harvested at the "partly opened" stage of maturation, that is when the outer petals were fully unfurled but the inner petals were still closed. Care was taken to maintain the transpiration stream by cutting the flower stalks under water. The flowers were used for the incorporation experiments within 15 min of harvest.

Introduction of Dibenzylethylenediamine (±)-Mevalonate-2-^{14}C into the Harvested Flowers

Use was made of the transpiration stream of the harvested flowers to introduce the radioactive precursors. Narrow-bore silicone-rubber tubing filled with tap water was attached to the cut ends of the flower stalks to form a watertight seal. 900 mμmoles of dibenzylethylenediamine (±)-mevalonate-2-^{14}C, 5·3 mc/mM (New England Nuclear Corp., 575 Albany Street, Boston, Mass., U.S.A.), in 0·05 ml water was added to the water in the silicone tubing just before it had been taken up completely. In some experiments 0·2 μmoles of sodium bicarbonate-^{14}C, 50 mc/mM, or 0·5 μmoles of sodium acetate-2-^{14}C, 50 mc/mM (Radiochemical Centre, Amersham, Bucks., England), in 0·05 ml water were used. The dibenzylethylenediamine (±)-mevalonate-2-^{14}C was washed in with three 0·01 ml portions of tap water, and the silicone-rubber tubing was then kept filled with tap water for the duration of the incubation period. The rate of transpiration varied and the uptake of the radioactive precursor took from 8–16 min. The rate of transpiration fell during the incubation period and was approximately half its initial rate after 3 hr incubation.

Incubation Conditions

Incubations with sodium acetate-2-^{14}C and (±)-mevalonate-2-^{14}C were carried out at 22° and at low light intensities (below 200 lux). Incubations with sodium bicarbonate-^{14}C were carried out at high light intensities in the conditions under which the plants were grown;[7] these latter conditions were chosen to maximize the possible contribution of label from $^{14}CO_2$ to the label found in the monoterpenes. The incubations were timed from the addition of radioactive precursor.

Extraction and Estimation of Petal Monoterpenes

3–4 g of petals were ground in dry ice, added to 8 ml of 0·1 M NaOAc buffer, pH 5·0, at 100° and immediately steam distilled for 10 min in an all-glass apparatus. The free monoterpenes were isolated from the steam distillates by extraction with 2·0 ml of redistilled 40–60° petroleum ether. The steam distilled residues were then extracted four times with 4·0 ml redistilled 40–60° petroleum ether, and heated to 80° for 15 min to remove residual traces of petroleum ether. 1 mg of β-glucosidase was added and the residues incubated for 3 hr at 40°. The monoterpenes released were extracted with 2·0 ml redistilled 40–60° petroleum ether.

The petroleum ether extracts were evaporated slowly in N_2 to a final volume of 35–40 μl. This was injected into a radio gas–liquid chromatograph.[20,21] Analysis for monoterpenes was performed on 2·75 m × 4 mm helical glass columns packed with 10% "free fatty acid phase" ("FFAP" Varian Aerograph, Walnut Creek, California, U.S.A.) on 100–120 mesh celite and temperature programmed from 100–250° at 1°/min with an argon flow rate of 60 ml/min. Geraniol and other monoterpenes were identified by comparison of their retention times with that of linalool and by co-chromatography with the appropriate standards (see also Ref. 7). Quantitation of radioactivity was made by comparison with standard ^{14}C methyl palmitate (10 μc/mM). The petroleum ether extracts of the steam distilled ground flower petals contained no free monoterpenes. 2-Phenylethanol, a major component of rose petals,[15,19] contained no radioactivity.

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