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BIOSYNTHESIS OF OLEANOLIC ACID GLYCOSIDES IN ISOLATED LIGULATE FLOWERS OF *CALENDULA OFFICINALIS*

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Key Word Index—*Calendula officinalis*, Compositae, oleanolic acid glycosides, labelling dynamics

Abstract—Three radioactive precursors, [3-^{3}H]-oleanolic acid and its 3-O-monoglucoside and 3-O-monoglucuronide, were administrated to ligulate flowers of *Calendula officinalis* The oleanolic acid was glycosylated to the monoglucoside and its derivatives faster than to the monoglucuronide and its derivatives After administration of each radioactive monoglycosides their hydrolysis to free oleanolic acid as well as further glycosylation was observed The 3-O-monoglucoside was hydrolysed more extensively than the 3-O-monoglucuronide and in both cases the 3-O-monoglucoside and its derivatives were formed faster than the derivatives of the 3-O-monoglucuronide

INTRODUCTION

The previous work on the biosynthesis of oleanolic acid glycosides in *Calendula officinalis* inflorescences employing the 3-O-monoglucoside and 3-O-monoglucuronide as precursors showed that both compounds were effectively absorbed by the inflorescences Radioactivity was found in the involucres as well as, after a delay, in the flowers in free oleanolic acid and in glycosides of both series as well as in unchanged precursor This result indicated the presence of active glycosidases and glycosylases in both the involucre and flowers However, these investigations did not resolve the problem of which compound was the main transport form from involucre to flowers and how it was metabolized by the flowers themselves

The aim of the present study was to examine the metabolism of radioactive [3-^{3}H]-oleanolic acid and its 3-O-monoglucoside and 3-O-monoglucuronide in isolated ligulate flowers of *Calendula officinalis*

RESULTS AND DISCUSSION

In the present work the [3-^{3}H]-oleanolic acid and its 3-O-monoglucoside and 3-O-monoglucuronide were used as precursors They were administrated to isolated ligulate flowers of *Calendula officinalis* After 2, 4, 6 and 8 hr flowers were analysed and the radioactivity was determined in free oleanolic acid and in oleanolic acid bound as the monoglucoside (I), the monoglucuronide (F) and in the total glycosides belonging to each series

Incorporation of radioactivity into these compounds after administration of [3-^{3}H]-oleanolic acid is presented in Fig 1

Oleanolic acid was effectively absorbed by the flowers The radioactivity in oleanolic acid decreased continually during the experiment The radioactivity was incorporated into all glycosides of series I and II and increased during the period of the experiment On the other hand in glycosides F and I, i e. in the precursors of both series, radioactivity was maintained at more or less the same level However, in I it was much higher than in F This indicates continuous glycosylation of oleanolic acid proceeding at a steady rate. Glycosides of series II were labelled three–four-fold more than the glycosides of series I The radioactivity incorporated into glycosides of series II was lower than that incorporated into their precursor (I), on the other hand, glycosides of series I were more labelled than their precursor (F) Since the total radioactivity incorporated into the glycosides does not compensate for the decrease of radioactivity in free oleanolic acid it is possible, that some 10% of the oleanolic acid undergoes degradation or transformation

Incorporation of radioactivity into different glycosides of oleanolic acid after administration of the 3-O-monoglucoside is presented in Fig 2

During all the periods investigated a decrease was observed in the radioactivity of the administrated precursor, i.e in glucoside I Simultaneously, the radioactivity in oleanolic acid increased markedly and after 8 hr it exceeded that in glycoside I This indicates that administrated precursor is extensively hydrolysed to free oleanolic acid The process of glycosylation of glucoside I proceeds at a much lower rate than the process of its hydrolysis, being most extensive during the first 2 hr Subsequently, the radioactivity present in glycosides of series II was constant which is similar to the situation in these glycosides after administration of free oleanolic acid (Fig 1) Glucuronide F and its derivatives, i e glucosides of series I, are labelled less than glycosides of series II Glucuronide F is less labelled than other compounds of this series which is similar to the results obtained after administration of free oleanolic acid

Changes in the radioactivity of different oleanolic acid glycosides after administration of radioactive 3-O-monoglucuronide are presented in Fig 3

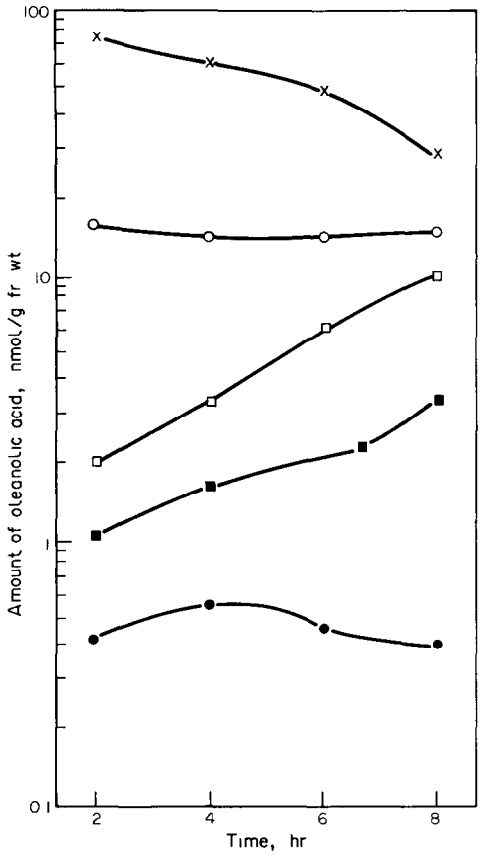

Fig 1 Radioactivity incorporated into free oleanolic acid (×), glucoside I (○), glycosides of series II (derivatives of I) (□), glucuronide F (●) and glycosides of series I (derivatives of F) (■) after administration of [3-^{3}H]-oleanolic acid

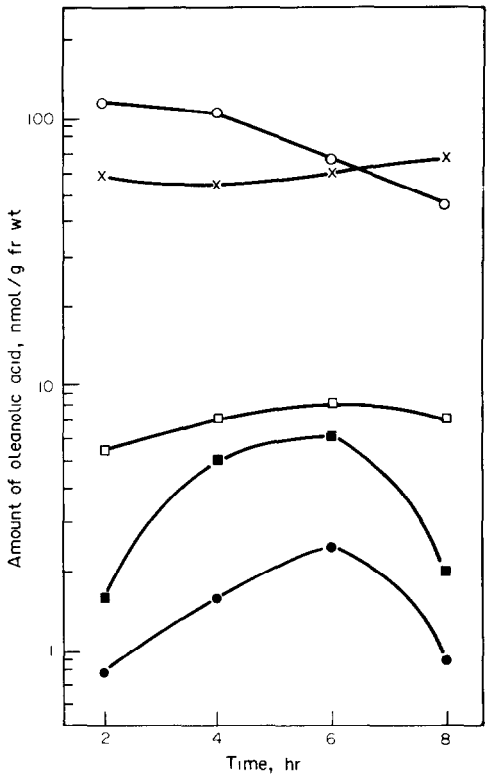

Fig 2 Radioactivity incorporated into free oleanolic acid (×), glucoside I (○), glycosides of series II (□), glucuronide F (●) and glycosides of series I (■) after administration of glucoside I

As in the case of administration of both previous precursors the radioactivity of precursor glucuronide F decreased However, its decline was lower than that observed when free oleanolic acid and glucoside I was used as precursors This indicates that the two first precursors are either metabolically more active or better absorbed compounds than glucuronide F In the flowers glucuronide F was metabolized mostly to glycosides of series I This process proceeded faster during the first 6 hr, and then stabilized after 8 hr The hydrolysis of glucuronide F to free oleanolic acid proceeded in parallel to glycosidation to derivatives of series I This process is slower than both the hydrolysis of I and also the process of glycosylation to series I The radioactivity in oleanolic acid increased for 6 hr but subsequently it stabilized, suggesting the partial utilization of oleanolic acid for the synthesis of glycosides of series II Labelling of glucoside I and its derivatives remained at a low level, but continually increased during the experiment

The present results on the dynamics of labelling of oleanolic acid glycosides in marigold ligulate flowers after administration of three precursors indicate the following conclusions Ligulate *Calendula officinalis* flowers are able to absorb and metabolize all three precursors From administrated precursors free

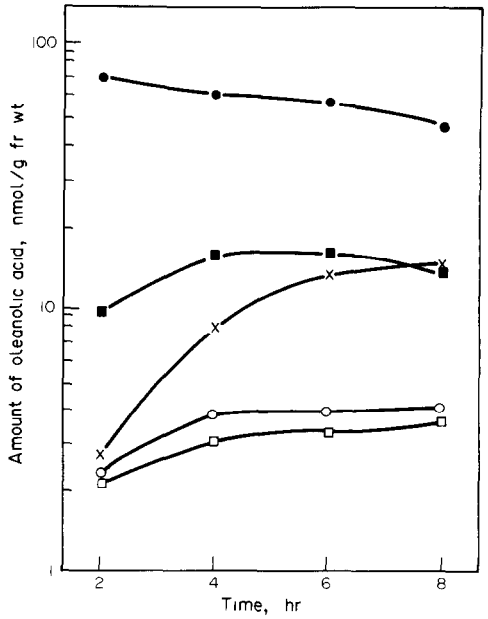

Fig 3 Radioactivity incorporated into free oleanolic acid (×), glucoside I (○) glycosides of series II (□), glucuronide F (●) and glycosides of series I (■) after administration of glucuronide F

oleanolic acid and all glycosides are formed but different rates of individual reactions are observed

The administrated oleanolic acid undergoes glycosylation to both monoglycosides (I and F), i e to precursors of both series The glycosylation of oleanolic acid to glucoside I predominates over that of glucuronide F Each of the monoglycosides is further glycosylated to members of its own series and the rate of the reaction increases during the period of the experiment The fact that glycosides are more extensively labelled than the glucuronides, in spite of their smaller quantity, confirms the results obtained for leaves of marigold by Janiszowska [1] and indicates faster metabolism of glycosides of series II in comparison to metabolism of glycosides of series I If the precursor administered to flowers was glucoside I, very rapid hydrolysis was observed Its hydrolysis dominated over its further glycosylation to glycosides of series II By analogy to the administration of oleanolic acid, the level of synthesis of glycosides of series I was low

After administration of glucuronide F as precursor, its hydrolysis was also observed However, this process was less extensive than hydrolysis of glucoside I. When inflorescences were used as the experimental material, faster hydrolysis of F was observed in the involucre, probably as the result of higher activity of the appropriate glycosidase

The results obtained allow the quantitative determination of the processes occurring in flowers after administration of precursors The metabolism of glycosylation to glycosides of the opposite series must proceed after hydrolysis to oleanolic acid The rate of glycosylation of oleanolic acid dominates over its glucuronidation Addition of glucose or glucuronic acid determines the sequence of further glycosylation steps to yield other glycosides

EXPERIMENTAL

Logulate flowers of *Calendula officinalis* var Radio were used for investigation All procedures of extraction, incubation and chromatography were the same as described in previous work

REFERENCE

1 Janiszowska, W and Kasprzyk, Z (1977) *Phytochemistry* **16**, 1919