Phytochemistry, 1972, Vol. 11, pp. 1733 to 1736. Pergamon Press. Printed in England.

SHORT COMMUNICATION

# INCORPORATION OF $CO_2$ INTO TRITERPENES IN YOUNG SHOOTS OF *HELIANTHUS ANNUUS*

KATHY STRÜBY, WIRGINIA JANISZOWSKA and ZOFIA KASPRYZK
Department of Biochemistry, University, Warszawa, Poland

(*Received* 26 *July* 1971)

**Abstract**—Studies of the rates of incorporation of $^{14}CO_2$ into triterpenoids in young shoots of sunflower revealed that the synthesis of five pentacyclic triterpene monols is followed by their hydroxylation to the corresponding diols as well as hydroxylation of oleanolic acid, formed by partial oxidation of β-amyrin, to echinocystic acid. Triterpene diols and echinocystic acid are further metabolized into unidentified compounds.

## INTRODUCTION

IT HAS been shown previously[1,2] that in all organs of sunflower during the whole vegetative period there occur 5 monols and 5 diols belonging to the pentacyclic triterpene series and two triterpene acids of β-amyrin group namely oleanolic acid[3] and echinocystic acid[4] (see Fig. 1). The level of all triterpenes in shoots and roots of sunflower is considerably

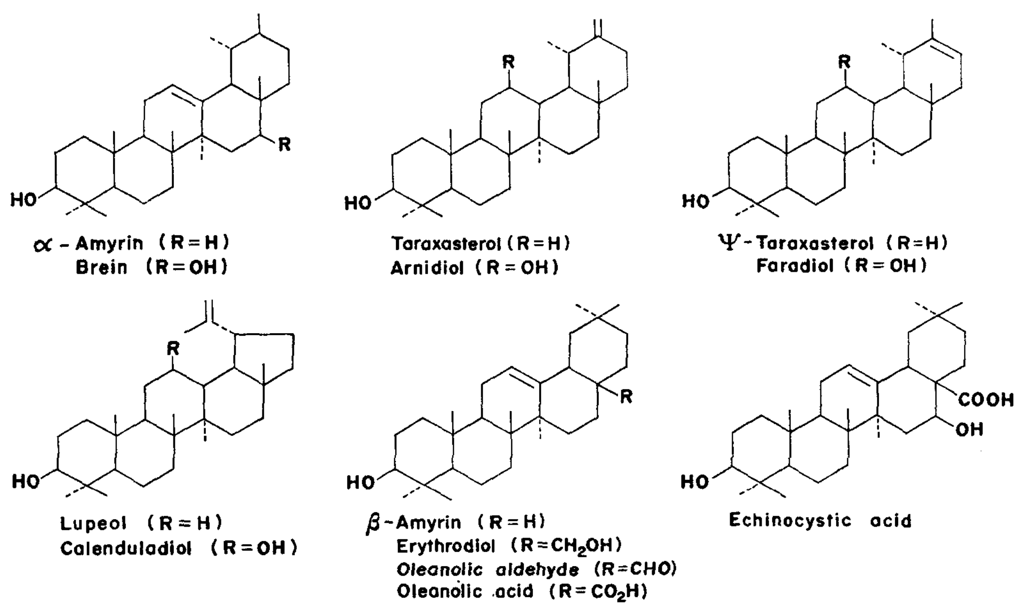


FIG. 1. TRITERPENES FROM *Helianthus annuus.*

[1] Z. KASPRZYK, K. STRÜBY and W. JANISZOWSKA, *7th International Symposium on the Chemistry of Natural Products, Riga 1970*, Abstracts, E 31, p. 446.
[2] Z. KASPRZYK and W. JANISZOWSKA, *Phytochem.* **11**, 1165 (1972).
[3] Z. KASPRZYK, Z. WOJCIECHOWSKI and I. KUCZEWSKA-JANKOWSKA, *Bull. Acad. Polon. Sci., Ser. sci. biol.* **14**, 747 (1966).
[4] W. JACHYMCZYK, Z. KASPRZYK, *Ann. Soc. Chim. Polon.* **36**, 15 (1962).

high,[5] which is different from *Calendula* previously studied by us. In *Calendula* various types of triterpenic alcohols occur exclusively in flower and seeds,[6,7] while the shoots and roots contain quantities of oleanolic acid and small amounts of its biogenetic precursors such as β-amyrin, erythrodiol and oleanolic aldehyde.[8]

Since the green parts of sunfliower are able to synthesize various types of pentacyclic triterpenes and catalyse their hydroxylation, studies were undertaken on the kinetics of their labelling. The preliminary results have been already published.[1]

## RESULTS AND DISCUSSION

Studies on the kinetics of labelling of triterpenes in the sunflower shoots were conducted using $^{14}CO_2$ as a precursor by the methods described previously.[9] The kinetics of labelling

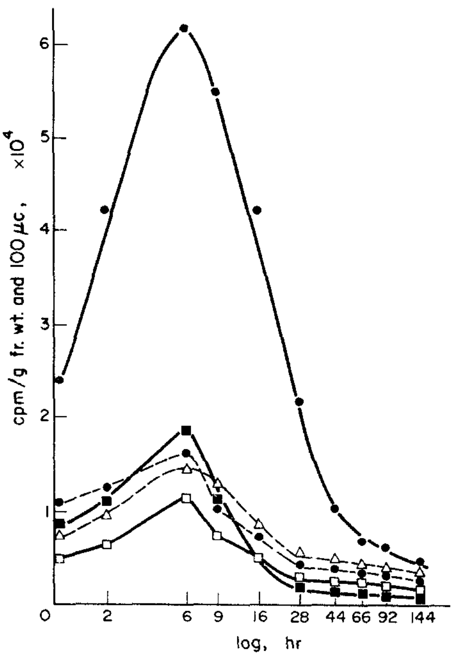


FIG. 2. THE KINETICS OF INCORPORATION OF $^{14}CO_2$ INTO TRITERPENOIC MONOLS IN YOUNG SHOOTS OF SUNFLOWER.

α-Amyrin (-- ●); β-amyrin (— ●); ψ-taraxasterol (□); taraxasterol (■); lupeol (△).

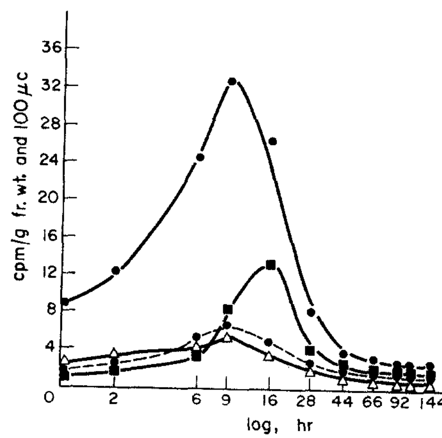


FIG. 3. THE KINETICS OF INCORPORATION OF $^{14}CO_2$ INTO TRITERPENIC DIOLS IN YOUNG SHOOTS OF SUNFLOWER.

Erythrodiol (— ●); brein (-- ●); faradiol with arnidiol (■); calenduladiol (△).

of triterpenic monols up to 144 hr after adding the precursor is presented in the form of graph in Fig. 2 and that of triterpenic diols in Fig. 3. The results were obtained using two different batches of plants. The most intensive labelling occurs with β-amyrin and erythrodiol, the intensity of labelling of other types of monols and diols being much lower.

[5] K. STRÜBY and Z. KASPRZYK, *Acta Soc. Bot. Pol.* **40**, 349 (1971).
[6] Z. KASPRZYK and M. FONBERG-BROCZEK, *Physiol. Plantarum* **20**, 321 (1967).
[7] Z. KASPRZYK, J. SLIWOWSKI and D. BOLESŁAWSKA-KOKOSZA, *Acta Biocheim. Polon.* **17**, 11 (1970).
[8] Z. KASPRZYK and Z. WOJCIECHOWSKI, *Phytochem.* **8**, 1921 (1961).
[9] Z. KASPRZYK, Z. WOJCIECHOWSKI and A. JERZMANOWSKI, *Phytochem.* **10**, 797 (1971).

The maximum of labelling of monols is observed at 6 hr and that of diols between 9 and 16 hr. This confirms the earlier suggested[1] precursor-product relationship between the monols and diols, i.e. between β-amyrin and erythrodiol, α-amyrin and brein, ψ-taraxasterol and faradiol, taraxasterol and arnidiol, and lupeol and calenduladiol. After reaching a maximum, the radioactivity of the diols decreases, implying the possibility of their further transformation.

The kinetics of labelling of β-amyrin derivatives is presented in Fig. 4. The results were obtained from experiments using four different batches of plants. The peak of labelling for β-amyrin, erythrodiol, oleanolic aldehyde, oleanolic acid and echinocystic acid occur at 6, 9, 28, 44, 92 and 144 hr respectively. The radioactivity of all compounds begins to drop after reaching maximal values. The results show: (1) in the sunflower shoots as in *Calendula*,

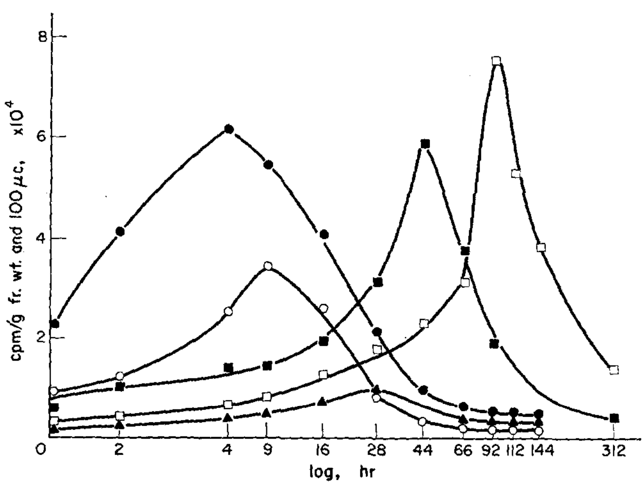


FIG. 4. THE KINETICS OF INCORPORATION OF $^{14}CO_2$ INTO TRITERPENES OF THE β-AMYRIN GROUP IN YOUNG SHOOTS OF SUNFLOWER.

β-Amyrin (●); erythrodiol (○); oleanolic aldehyde (▲); oleanolic acid (■); echinocystic acid (□).

oleanolic acid is formed as a result of oxidation of 28-CH group of β-amyrin through erythrodiol and oleanolic aldehyde as intermediates; (2) echinocystic acid is formed by hydroxylation from oleanolic acid; and (3) echinocystic acid is further metabolized to unidentified products.

The metabolism of β-amyrin is different in sunflower from that in *Calendula*. In both plants the labelling curves in the initial stage indicate a precursor-product relationship between β-amyrin and oleanolic acid. On the other hand, labelling of intermediate compounds such as erythrodiol and oleanolic aldehyde in *Calendula* is very low, thus indicating the continuous oxidation of β-amyrin to oleanolic acid. By contrast, in sunflower labelling of erythrodiol is relatively high, suggesting that hydroxylation of β-amyrin to erythrodiol is mediated by the same enzyme that hydroxylate the other monols to diols. Further oxidation of erythrodiol to oleanolic acid occurs probably later on using different enzyme systems. The final product of oxidation of β-amyrin, oleanolic acid, in *Calendula* is very slowly metabolized since no decrease in radioactivity was observed even 500 hr after addition of radioactive acetate. On the other hand, in sunflower the radioactivity of oleanolic acid after reaching its maximum (at 44 hr), rapidly decreases both after $CO_2$ and acetate feeding.[1] This can be explained in terms of its transformation to echinocystic acid, the radioactivity of which simultaneously increases. This acid is also further metabolized, since its radioactivity after reaching a maximum also rapidly diminishes. The results obtained indicate that the metabolism of triterpenes in young shoots of sunflower is reasonably fast.

## EXPERIMENTAL

*Material.* Plants of *Helianthus annuus* were cultivated in a phytotron under conditions described previously.[6]

*Administration of* $^{14}CO_2$. Plants (3-week-old) with partially removed leaves 500 g, located separately in chambers of Plexiglass, were treated with 50 $\mu$C of $^{14}CO_2$, which was released from $Na_2\,^{14}CO_3$ by means of $H_2SO_4$. The plants were illuminated during 1 hr with light of intensity 40 000 lx. During that time the plants incorporated 95% of the administered $^{14}CO_2$. Then the plants were kept outside of the chambers in vessels with tap water with 3000 lx illumination during an 8-hr day. The samples for analyses were taken at various time intervals after removal from a chamber.

*Fractionation of material* was conducted as described previously.[8] Oleanolic aldehyde was determined as erythrodiol after reduction with $NaBH_4$.

*Radioactivity was measured* in 5 ml of toluene containing 15 mg PPO and 1·5 mg POPOP in the scintillation counter USB2 (made in Poland) with 60% efficiency.

*Key Word Index*—*Helianthus annuus*; Compositae; biosynthesis; triterpenoids; incorporation of $^{14}CO_2$.