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TRANSLOCATION OF SITOSTEROL* AND RELATED COMPOUNDS IN *PELARGONIUM HORTORUM* AND *HELIANTHUS ANNUUS*†

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Key Word Index—*Pelargonium hortorum*; Geraniaceae; *Helianthus annuus*; Compositae; sterol translocation; sitosterol; sterols; sitosteryl glycoside; triterpenes; water soluble sterols.

Abstract—The translocation of several plant sterols and a triterpene was studied in geranium and sunflower plants. Upward translocation of sitosterol-[^{14}C] and β-amyrin-[^{14}C] was shown within 48 hr to the upper parts of a geranium plant sectioned previously above the roots. Downward translocation of sitosterol-[^{14}C] from the leaf of application was evident in intact plants after 48 hr. In addition to free sitosterol-[^{14}C] considerable amounts of sitosteryl-[^{14}C] glycoside and traces of sitosteryl-[^{14}C] ester were found in most parts examined. Very slow downward translocation of cholesterol-[^{14}C] but not of desmosterol-[^{14}C], sitosteryl-[^{14}C] palmitate or β-amyrin-[^{14}C] was shown in geranium. In sunflower no downward translocation of cholesterol-[^{3}H], sitosteryl-[^{3}H] acetate or palmitate could be detected. In geranium, sitosteryl-[^{14}C] glycoside translocated downward from the leaf of application to all other plant parts, except other leaves, and was found in these parts after 10 days as the unchanged glycoside, free sterol and steryl ester. The effect of drying the plant parts on the recovery of radioactive steroidal material is discussed. Traces of a water soluble, dialyzable form of sterol-[^{14}C] were also detected in dried geranium roots after treatment with strong acid or alkali.

INTRODUCTION

The experiments described here were primarily designed to determine the pathway of sitosterol-[^{14}C] movement following its application to a geranium (*Pelargonium hortorum*) leaf. Sitosterol was initially chosen because of its ubiquitous distribution in nature, and geranium was chosen because of its availability throughout the year, because its large, relatively flat leaves readily absorb Tween 80–H_2O–lipid solutions and because sitosterol and β-amyrin are the principal sterol and triterpene respectively of this plant [2]. During the course of this work the question of possible sitosteryl glycoside biosynthesis arose, and became more pertinent when the glycoside was actually isolated from the plant.

RESULTS

Upward translocation of sitosterol-[^{14}C] *and* β*-amyrin-*[^{14}C] *in geranium*

Both sitosterol-[^{14}C] and β-amyrin-[^{14}C] were readily translocated to all leaves when administered to the cut portion of the lower main stem (sectioned 1 cm above the soil) of an intact geranium plant. The distribution of sitosterol-[^{14}C] was the same with respect to both time and extent of distribution of sterol. It was evident that the distribution of labeled sterol and triterpene throughout the plant tissues was quite thorough

* We are in agreement with Nagasampagi *et al.* [1] that the prefix β for sitosterol is no longer necessary.

† A preliminary report was presented at the American Society of Plant Physiologists, Memphis, Tennessee, 4 and 5 February 1974.

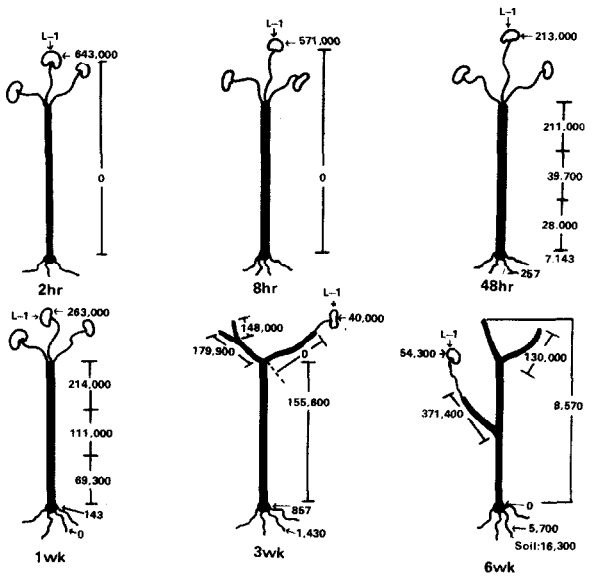

Fig. 1. Downward translocation of sitosterol-[^{14}C] in *Pelargonium hortorum*. L-1 = leaf to which the radioactive marker was applied.

as shown by autoradiography. No sequential time period studies were performed, but both sterol and triterpene were translocated to the top leaves within 48 hr.

Downward translocation of sitosterol-[^{14}C] (methods A and B)

Figure 1 shows that sitosterol-[^{14}C] placed on an upper leaf (hereafter referred to as L-1) as a Tween 80–H_2O preparation was slowly translocated to all other parts of the plant except the other leaves. The process appeared to be limited to the stem and root system only. In this particular series of experiments (Method A), column chromatography, TLC and GLC-RC, showed that the radioactivity of the "neutral" fraction appeared to be in unchanged sitosterol-[^{14}C]. In later experiments, (Method C) when larger quantities of sterol-[^{14}C] were administered, small quantities of labeled steryl ester were detected in most plant parts. During this work, the possibility arose that labeled sterol might be excreted into the soil. For example, more ^{14}C was detected in the soil as Et_2O soluble material than could be accounted for by contamination with rootlet parts. This ^{14}C-material co-chromatographed with sitosterol on TLC. In fact at the 6 week period no radioactivity was detected in the root, but labeled ether soluble material (16300 dpm) was detected in the surrounding soil. The possibility of excretion into the soil was also suggested by decreasing recovery of labeled sterol from the total plants after prolonged experimental periods.

Data for the extensive set of experiments described in Method B, in which tissues were dried prior to extraction, will not be presented since only in the case of L-1 could any ^{14}C-label be extracted by organic solvents or H_2O. The only positive data obtained, from one set of the experiments described, is presented in Table 1.

Experiments on the H_2O-soluble sterol released by acid hydrolysis from the root system indicated that this material was dialyzable and presumably has a molecular weight of less than 10000. The same result was found with the labeled material that was secreted into water from a geranium plant suspended in H_2O (Table 2) and with that released into the soil from intact geranium plants presented with sitosterol-[^{14}C] via L-1 (0 dpm after 24 hr; 7000 dpm after 48 hr; 700 dpm after 3 days and 700 dpm after 1 week). With the intact plant growing in soil the problem is considerably more complicated by the fact that soil bacteria and fungi may metabolize the sterol as rapidly as it is excreted or may convert secreted H_2O-soluble sterol to the free form or vice versa. This

Table 1. Label extracted by various solvents from dried plant parts 1 week after administration of sitosterol-[^{14}C] to a single leaf (L-1) of *Pelargonium hortorum*

Plant part	Hexane	Acetone	Ethanol (dpm × 10^{-3})	H_2O	H_2O-soluble after acid treatment of solvent-extracted tissue
L-1	120	100	16	0·70	0
Upper main stem	0	0	Trace†	0	0
Lower main stem	0	0	Trace	0	8·0
Root	0	0	Trace	0	11·0
Rootlets	0	0	Trace	0	0

* L-1 was presented with 640 × 10^3 dpm of sitosterol-[^{14}] (Method B; Experimental) for 1 week. The plant was then sectioned and the individual parts dried for 1 week before the extractions were performed. † Trace = 0·1–0·5 × 10^3 dpm.

Table 2. Detection of H_2O-soluble ^{14}C excreted from the roots of 9 geranium plants alternately suspended in H_2O and plant food solution*

Steep†	Day of collection‡	Total H_2O-soluble ^{14}C-material dpm × 10^2
1st H_2O	2	6
1st Rapid Gro	4	4
2nd H_2O	6	6
2nd Rapid Gro	8	6
3rd H_2O	11	0·8
3rd Rapid Gro	17	4
4th H_2O	24	6
4th Rapid Gro	32	8
4th H_2O	37	4
4th Rapid Gro	40	10
5th H_2O	42	8
5th Rapid Gro	44	6
6th H_2O	48	4
6th Rapid Gro	50	8

* 5 μCi of sitosterol-[^{14}C] was placed on L-1.

† One liter of H_2O or 1·7 g of Ra-Pid-Gro per liter of distd H_2O.

‡ Following administration of sitosterol-[^{14}C] to L-1.

is illustrated by an experiment in which 4 μCi of sitosterol-[^{14}C] was allowed to stand in potting soil for several weeks. The potting soil was then sequentially extracted with hexane once and acetone twice. The radioactivity recovered in each extraction was 100000, 260000 and 68000 dpm, respectively. No attempt was made in this experiment to determine whether H_2O-soluble sterol had been formed. It has been well established that soil bacteria and fungi are capable of transforming plant sterols to other substances [3].

Translocation of free sitosterol-[^{14}C] and sitosteryl-[^{14}C] glycoside (method C)

After discovery of the sterol "binding" effect induced by drying of the plant parts following the administration of sitosterol-[^{14}C] to L-1, additional experiments were performed in which the plant parts were immediately placed in boiling ethanol and treated as described in Method C. Aliquots of the ethanol extract were analyzed for free sterol and steryl ester and the results are shown in Fig. 2. Large amounts of free sitosterol-[^{14}C] and its glycoside, but only trace amounts of sitosteryl-[^{14}C] ester were found in the plant parts examined. No ^{14}C was found in any leaves other than L-1. Aliquots of the EtOH extracts were also examined for steryl glycoside but because of lack of suitable reference standards, examination for labeled acylated sitosteryl glycoside could not be made. The possibility that a considerable amount of sitosterol-[^{14}C] administered to L-1 might not be absorbed into the plant was tested. The amount not absorbed varied from 8 to 24% in five experiments. Thus most of the sitosterol presented to L-1 was indeed absorbed into the leaf.

The distribution of ^{14}C into various plant parts following administration of biosynthetically derived sitosterol-[^{14}C] glycoside is shown in Fig. 2. Translocation of sitosteryl-[^{14}C] glycoside in geranium was accompanied by conversion of at least part of the glycoside to free sterol and steryl ester. Whether this glycoside cleavage occurs prior to, during, or after translocation of the sterol, or a combination of all three, has yet to be determined. Recovery of total radioactivity by solvent extraction was low for reasons we cannot explain at present.

Failure of translocation of other labeled compounds from L-1 in geranium and sunflower

Examination of all plant parts by method A (for geranium) and method C (for sunflower), indi-

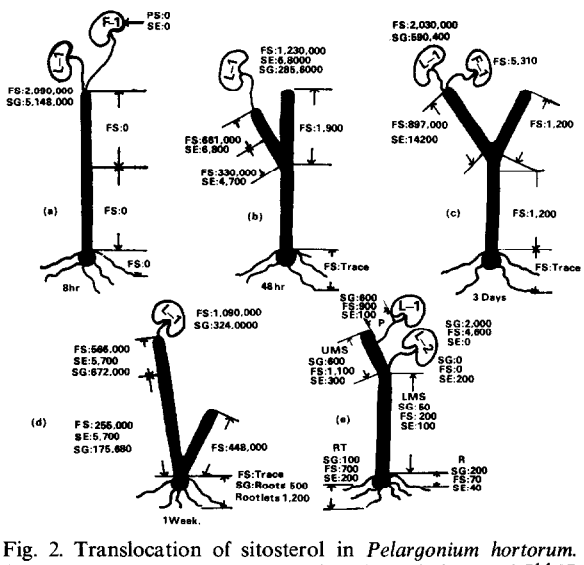

Fig. 2. Translocation of sitosterol in *Pelargonium hortorum*. A, B, C and D: downward translocation of sitosterol-[^{14}C] (Method C) in a geranium plant within one week. No translocation was evident at 24 hr; E: downward translocation of sitosteryl-[^{14}C] glycoside in a geranium plant after 10 days. L-1 = leaf to which the radopactove marker was applied; P = petiole; L-2 = leaf 2; UMS = upper main stem; LMS = lower main stem; R = root; RT = rootlets; Fl = flower; FS = free sterol; SE = steryl ester; SG = steryl glycoside.

cated that no downward translocation of sitosteryl palmitate, β-amyrin or desmosterol occurred in geranium, or cholesterol, sitosteryl acetate or palmitate in sunflower. However, on re-inspecting our data on translocation of cholesterol-[26-^{14}C] in geranium we noted that after 14 days some 154000 dpm had migrated to the upper portion of the main stem (approximately 10 cm in length) to which L-1 was attached. We are reluctant to describe this as translocation of cholesterol at this time since: (1) the original labeled cholesterol was not subjected to TLC before use (a contaminant may have migrated); (2) the 26-^{14}C may have been metabolized and subsequently converted to another compound; and (3) no translocation of ^{3}H-labeled cholesterol in sunflower was detected. It appears that from these results that translocation of sterols (and triterpenes) must in part depend upon some specific structural characteristics.

DISCUSSION

Our experiments show that β-amyrin-[^{14}C] and sitosterol-[^{14}C] can be transported upward via xylem. The relevance of these observations to physiological translocation is open to question. The upward translocation might be due to capillary action through the xylem and might also involve phloem translocation. In any case, since it appears likely that a hydrophobic lipid-like sitosterol would move freely in an essentially aqueous medium, a protein carrier loosely bound to the sterol is implied. The observed downward (phloem) translocation of sitosterol also cannot be fully assessed at this time. It seems especially puzzling that sitosterol should be translocated in geranium in view of the fact that all morphologically distinct parts of the mature plant are capable of synthesizing sitosterol (unpublished results).

Sitosterol is converted to several other compounds by higher plants [4] but in our experiments the plant tissues were not examined for trace steroid metabolites. Sitosteryl ester can be eliminated as a translocatable form of the sterol. Only minute traces of steryl ester were found in other parts of geranium following administration of the free sterol to L-1. In addition, sitosteryl palmitate (in geranium) and the acetate and palmitate (in sunflower) were not translocated when administered in a similar manner.

Sitosteryl glycoside could not be positively ascertained as a translocatable form of sterol. When administered to L-1, the glycoside was found in good quantity in the main stem and in traces in the root system, along with free sterol and steryl ester. However, the original glycoside could have been hydrolyzed in L-1, then transported as such to other plant parts to re-form glycoside and steryl ester *in situ*. Although non-absorption of β-amyrin, free cholesterol, desmosterol, sitosteryl acetate and palmitate into the leaves is possible, it seems highly improbable since all substrates were administered in the same way as sitosterol. Evidence that sitosterol can be excreted into the soil in a H_2O-soluble form has been presented and this is the only explanation we have for the generally poor total recovery of ^{14}C-labeled sterol. It seems unlikely that the free sterol would be excreted as such, and possibly the H_2O-soluble form released by strong acid or alkali treatment is involved. However, the latter was not found in any plant part isolated by Method C, thus in effect eliminating this possibility. Formation of one H_2O-soluble form seems to be associated with the phenomenon of drying.

The remarkable stability of the "H_2O-solubilized sterol complex" liberated by strong acid and/or alcoholic KOH from dried plant tissue suggests that it is not identical with any of the previously detected H_2O-soluble plant sterols [5–8]. We use the term "sterol complex" with some reservation since we have no proof that the compound contains the original intact sitosterol nucleus. However, since the sitosterol was initially labeled at C-3 it seems probable that a large portion of the molecule is intact. Although the nature of the H_2O-solubilizing component is unknown, an ether linkage with the sterol portion is suggested by the stability of the compound. Since both the root-released form and that obtained by hydrolysis of the dried root system are dialyzable, they must have a molecular weight less than 10000 and may be identical.

Our results are in general agreement with those of other workers who have studied steroid and triterpenoid translocation in plants [9–15] but it is clear that many aspects require further experimentation.

EXPERIMENTAL

Plant material. Potted geranium plants (*Pelargonium hortorum*) were purchased locally. They usually consisted of mature plants, occasionally in flower, and they were all the same cultivar of this highly inbred species. Sunflowers (var. Mammoth) were grown in the field and the compounds investigated were applied to mature flowering plants.

Chemicals and radioactive substrates. All solvents were distilled before use. Sitosterol-[3-^{14}C] (5·17 mCi/mM) was purchased from N. V. Philips-Duphar, Holland. Desmosterol-[26-^{14}C] (45 mc/mM), and cholesterol-[26-^{14}C] (45 mc/mM) and mevalonic acid-[2-^{14}C] (5·8 mc/mM) were purchased from New England Nuclear Corp., Boston, Massachusetts. β-Amyrin-[^{14}C] (3·28 × 10^5 dpm/mg) was biosynthesized from mevalonic acid-[2-^{14}C] as described by Baisted *et al.* [16]. Sitosteryl-[3-^{14}C] palmitate was synthesized by refluxing (30 min) under anhydrous conditions the following mixture: 5·14 μCi of sitosterol-[3-^{14}C], 10 ml dry pyridine and 2 ml palmitoyl chloride. Cooled mixture was diluted with H_2O and extracted with Et_2O. The Et_2O extract was washed successively with 10% HCl, 5% NaOH and 3× with distilled H_2O, followed by removal of the Et_2O on a steam bath under N_2. The ester was purified by chromatography on an alumina column and TLC. Sitosteryl-[22,23-^{3}H(N)] acetate was synthesized from sitosterol-[22,23-^{3}H(N)] (32·0 Ci/mM). The latter and cholesterol-[1,2-^{3}H(N)] (52·2 Ci/mM) were purchased from New England Nuclear Corp. Sitosteryl-[3-^{14}C] glycoside was isolated and purified from a geranium plant to which sitosterol-[3-^{14}C] had been administered via L-1, Sitosteryl glucoside was synthesized by the method of Gisvold [17] from sitosterol (generously supplied by the Upjohn Laboratories, Kalamazoo, Michigan) consisting of 55% sitosterol, 7% stigmasterol and 38% campesterol as determined by GLC. After several crystallizations from hot EtOH and EtOH–pyridine the glucoside gave mp 294–300° (lit. [18, 19] mp 298°, 285–290°).

General methods. The solvent system used for TLC of free sitosterol (R_f 0·3) and sitosteryl esters (R_f 0·95) was petrol–Et_2O (7:3, solvent system A). TLC of steryl glycoside (R_f 0·25) used *n*-BuOH–HOAc–H_2O (4:1:1, solvent system B). All free and combined sterols were eluted from silica gel by centrifugation with warm pyridine. Visualization of TLC standards was by spraying with anisaldehyde reagent. Autoradiograms were prepared by superimposing X-ray film (Kodak, RP's X'omat RPS-54) on a glass plate on which the plant leaf was placed, with pressure applied. After 3 weeks exposure in the dark, the films were developed. Radioactive determinations of lipid extracts were performed as previously described [20]. Aqueous samples to be examined for radioactivity were counted in Insta-gel emulsifier (Packard Instrument Co., Downers Grove, Ill.)

Isolation of sitosteryl glycoside from whole geranium plant. 1400 g of dried and finely ground whole geranium plant was exhaustively extracted with petrol followed by extraction with hot Me_2CO for 2 days. The Me_2CO extract was concentrated and left for 1 week at room temp. The residue was re-crystallized several times from hot Me_2CO and yielded a crystalline product (120 mg; mp 290–300° decomp.) which cochromatographed on TLC with synthetic sitosterol glucoside. Acid hydrolysis of the crystalline compound in 1·5 N HCl yielded a free sterol identified by TLC and GLC as sitosterol (98%), mp 137–139°, with small amounts of campesterol and stigmasterol.

Application of radioactive substrates to the plants. Each of the labeled substrates was dissolved in 0·5 ml of Me_2CO. Two drops of Tween 80 were added followed by 0·5 ml H_2O. The mixture was heated under a stream of N_2 until a vol of 0·5 ml was reached and the odor of Me_2CO was no longer noticeable. A clear soln, readily absorbed by the plant was obtained. For upward translocation experiments in geranium the plant was cut within 1 cm of the soil and the stem section immediately placed in a small vessel containing the labeled substrate soln. After the labeled material was absorbed (usually 2 hr or less) sufficient H_2O was added to maintain the plant's viability during the experimental period. For downward translocation experiments the substrate soln was placed on the surface of a single upper leaf (L-1) of the plant and allowed to be absorbed slowly.

Treatment of plant material with sitosterol-[^{14}C] (*methods A, B and C*). One plant in each group was presented with 640000 dpm of sitosterol-[^{14}C]–Tween 80–H_2O preparation applied to the periphery of L-1 only. The other plants of each "light group" had the labeled sitosterol applied to the inner lighter green area no more than 2 cm from the petiole to which the leaf was attached. Lighting conditions for method C were essentially the same as for method A. Other differences in experimental conditions between methods A, B and C were as follows: *Method A.* Tissues were immediately boiled in EtOH, dried and extracted with the same solvent for 24 hr. A neutral fraction was prepared by partition between Et_2O and 5% KOH soln. No Et_2O soluble acidic compounds could be detected. *Method B.* Tissues were allowed to dry before extraction then ground and extracted with hexane (24 hr), Me_2CO (24 hr), EtOH (72 hr) and H_2O (48 hr). *Method C.* The same as Method A except that wet tissues were ground and extracted with EtOH (72 hr) then with hot H_2O (24 hr). This was followed by hydrolysis of the tissues with conc. HCl–H_2O (3:1) and the hydrolysate was examined for "H_2O solubilized" ^{14}C-label. One-fourth of the EtOH extract was examined for glycosides and acylglycosides, the remainder was converted into a neutral fraction.

Treatment of solvent and H_2O *extracted plant parts with conc* HCl *or alcoholic* KOH. Dried and solvent-extracted plant parts (Method B) from which little or no ^{14}C-label had been recovered were refluxed for 8 hr with 75 ml conc HCl and 25 ml H_2O. The cooled mixture was extracted with 300 ml Et_2O; no ^{14}C was detected in this extract and it was discarded. The aqueous phase was evaporated to dryness and assayed for radioactivity. In one case a lower main stem was refluxed for 2 hr in EtOH–C_6H_6–H_2O (4:5:5) with 25% (w/v) KOH, the mixture used to saponify steryl esters. After diln with H_2O and extraction with Et_2O the aqueous phase was evaporated to dryness and examined for radioactivity.

Examination of steryl ester fractions. Steryl ester fractions were separated and purified by TLC in solvent system A. The recovered esters were saponified and all the radioactivity was detected in the free sterol.

Detection of sitosteryl-[^{14}C] glycoside. In experimental method C, the EtOH extract residues were dissolved in hot C_5H_5N* and one quarter was used for glycoside-[^{14}C] determination. The remainder was freed of C_5H_5N and converted to neutral fractions as described for Method A. Free sterol and steryl ester were separated from steryl glycoside by TLC using the solvent system A; steryl glycoside remained at the origin. The steryl glycoside regions were extracted with warm pyridine and rechromatographed in the same system for further purification. They were then subjected to TLC in sol-

* Sitosteryl glycoside is very insoluble in most organic solvents and practically insoluble in H_2O. The best solvents in increasing order are: hot $CHCl_3$–MeOH (2:1), C_5H_5N and Me_2CO.

vent system B. In experiments involving application of sitosteryl-[^{14}C] glycoside to L-1, the plants were sectioned after 10 days and immediately extracted with hot EtOH, followed by $CHCl_3$–MeOH (2:1) for 24 hr. The combined extracts were dried under *vacuo* and the residues subjected to TLC as described above.

Isolation of ^{14}C-labeled compounds from the soil following 6 weeks application of sitosterol-[^{14}C] to a single geranium leaf. The soil from the 6 week application of sitosterol-[^{14}C] to L-1 was covered with H_2O, stirred and the H_2O decanted. This was repeated until no floating debris was evident. The soil was then ground and extracted 48 hr with EtOH in a Soxhlet extractor. The EtOH was removed by distn and residue partitioned between Et_2O and 5% KOH. The Et_2O extract was washed with H_2O and residue subjected to TLC (solvent system A). In other experiments (Method C) the soil was allowed to dry following the experimental period, saturated with 1 liter H_2O and filtered. The filtrate was evaporated to dryness and residue examined for radioactivity.

Dialysis of H_2O-soluble fractions. Portions (30–40 ml) of the H_2O-soluble ^{14}C-labeled material were placed in 2·2 cm diameter cellulose dialyzing tubing (A. H. Thomas Co., Philadelphia) and dialyzed in 400 ml distd H_2O at 4°. The H_2O was changed each day so that 4 dialysate fractions were collected. These, and the material remaining in the dialyzing tube, were evaporated to dryness and the residues assayed for ^{14}C. Materials examined in this manner included the H_2O-soluble material obtained after acid treatment of dried root system (Table 1), adjusted to pH 7 before dialysis; the H_2O-soluble material obtained from soil; and the H_2O-soluble material obtained from a geranium plant suspended in H_2O (Table 2).

Recovery of ^{14}C-labeled compounds from soil incubated with sitosterol-^{14}C. A mature geranium plant was removed from its pot and the soil gently shaken from the root system. A portion of soil was removed from the center of the pot and 4·0 μCi of sitosterol-[^{14}C] in 0·5 ml C_6H_6 was placed in the groove, which was then covered with the soil removed. The pot was allowed to stand undisturbed at room temp. with ordinary laboratory light conditions for 6 weeks and watered once a day. Soil was then pulverized and extracted for 24 hr with hot hexane and 2× for a similar period with Me_2CO. Solvent from each extraction period was boiled off and residue examined for radioactivity.

Recovery and dialysis of H_2O-soluble ^{14}C-sterol from a geranium plant with root and rootlets suspended in H_2O. Sitosterol-[^{14}C] (5 μCi) as a Tween 80–H_2O preparation was placed on L-1 of a large geranium plant mounted on a ring stand with its roots just covered with H_2O (about 1 liter). At intervals over a period of about 6 weeks the H_2O was alternated with an equal vol of a soln containing 1·7 g/l of Ra-Pid-Gro soluble plant food (Ra-Pid-Gro., Danville, N.Y.). The "steeps" were reduced to dryness at 60° and residues examined for ^{14}C content. At the end of the experimental period the root was practically decomposed, but several leaves were still green and the stems were mainly green and viable. Portions of two H_2O and two Ra-Pid-Gro "steeps" collected during the third and fourth week were combined. The combined sample, containing 600 dpm of ^{14}C was subjected to dialysis as described above.

Application of other labeled sterols and derivatives to L-1 of geranium or sunflower. The following compounds were added to L-1 of individual geraniums and the plant examined for translocation, following the periods indicated, by Method A: $7{\cdot}14 \times 10^5$ dpm cholesterol-[26-^{14}C] (14 days); $7{\cdot}14 \times 10^5$ dpm desmosterol-[26-^{14}C] (14 days); $7{\cdot}14 \times 10^5$ dpm sitosterol-[3-^{14}C] palmitate (2 days, 1 and 3 weeks); $5{\cdot}30 \times 10^5$ dpm β-amyrin-[^{14}C] (2 days, 1 and 3 weeks); $1{\cdot}01 \times 10^5$ dpm sitosteryl-[3-^{14}C] glycoside (10 days).

The following compounds were similarly added to L-1 of individual sunflower plants which were examined for translocation in the same manner: $2{\cdot}22 \times 10^9$ dpm cholesterol-[1,2-^{3}H(N)] (25 days); $3{\cdot}1 \times 10^8$ dpm sitosteryl-[22,23-^{3}H(N)] acetate (30 days); $5{\cdot}4 \times 10^8$ dpm sitosteryl-[22,23-^{3}H(N)] palmitate (30 days).

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