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ALDOXIMES AS INTERMEDIATES IN THE BIOSYNTHESIS OF TYROSOL AND TYROSOL DERIVATIVES

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Abstract—Aldoximes derived from amino acids by *N*-hydroxylation are found to be precursors of tyrosol, *p*-methoxyphenethyl alcohol and their glycosidic derivatives in higher plants. In *Sinapis alba* *p*-hydroxyphenylacetaldehyde oxime is converted into tyrosol with a much higher rate than L-tyrosine. Similarly, in *Aubrietia* species the biosynthesis of *p*-methoxyphenethyl alcohol from L-*p*-methoxyphenylalanine proceeds via *p*-methoxyphenylacetaldehyde oxime. Only 0·05% of the radioactivity of labelled L-*p*-methoxyphenylalanine was incorporated into *p*-methoxyphenethyl alcohol while more than 1% of the oxime was converted into the alcohol and its glucoside. As no transformation of *p*-hydroxyphenylacetaldehyde oxime into *p*-methoxyphenethyl alcohol was detectable the *O*-methylation obviously takes place at the level of the amino acid. The structure of a tyrosol derivative formed after feeding of *p*-hydroxyphenylacetaldehyde oxime-1-^{14}C was elucidated; methylation and enzymatic cleavage with purified β-glucosidase yielded only *p*-methoxyphenethyl alcohol. This indicates that the hydroxyl group of the C_2-side chain of tyrosol is bound to D-glucose by a β-glycosidic linkage. *p*-Hydroxyphenylacetaldehyde oxime as well as *p*-methoxyphenylacetaldehyde oxime arise from the corresponding amino acids in the plants investigated. The significance of aldoxime formation and metabolism in various plants is discussed.

INTRODUCTION

THREE years ago it became known that aldoximes are constituents of higher plants and are metabolites of amino acids.[1,2] This conversion, which takes place both with aromatic and aliphatic compounds, also includes non protein amino acids such as homomethionine and phenylbutyrin.[1-3] Recently, Kindl and Underhill[4,5] obtained evidence that *N*-hydroxylation of the amino acids followed by oxidative decarboxylation of the *N*-hydroxyamino acids is involved in the process.

It is now well established that aldoximes are precursors of mustard oil glucosides (VI, Scheme 1)[1,3,6] and cyanogenic glycosides (IV, Scheme 1);[7] it was also tempting to speculate that other nitrogen compounds such as nitro derivatives may be formed from aldoximes.[6,8]

While studying the biosynthesis of *S*-(β-D glucopyranosyl)-*p*-hydroxyphenylacetothiohydroxamic acid (*V*, Ar = *p*-hydroxyphenyl) and related compounds we made the surprising observation that labelled aromatic aldoximes with a C_6-C_2-skeleton were converted in high percentage into glycosides which behave during chromatography very similarly to

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the expected thiohydroxamic acid derivatives. However, subsequent acid hydrolysis did not lead to derivatives of phenylacetic acid but yielded phenethyl alcohols, e.g. tyrosol (IX, Ar = *p*-hydroxyphenyl). Such compounds have been found only very rarely in higher plants so far. Veer *et al.*[9] obtained tyrosol from *Ligustrum ovalifolium*, while Troshchenko and Kutikova[10] reported its occurrence in *Rhodiola* species; also certain berries seem to accumulate this compound.[11–13] More frequently, phenethyl alcohol and its *p*-hydroxy derivatives appear as products of various fermentations.[14,15] Furthermore, the pathway L-tyrosine (I) → *p*-hydroxyphenylpyruvic acid (VII) → *p*-hydroxyphenylacetaldehyde (VIII) → tyrosol (IX, Ar = *p*-hydroxyphenyl) has been described in yeasts[16] and seems likely to occur in tubercle bacteria.[17] In contrast to this reaction sequence in bacteria and yeasts, higher plants seem to have a different biosynthetic route to tyrosol. This finding and the determination of the structure of the tyrosol derivatives isolated from higher plants are the subject of this paper.

RESULTS

Biosynthesis of p-*Methoxyphenethyl Alcohol and its Glucoside*

During the course of experiments concerning the metabolism of aromatic aldoximes in higher plants, the formation of arylethanol derivatives was observed. As these alcohols can reasonably be assumed to be biosynthesized by a route involving aldoximes, investigations were started on the biosynthesis of the most stable compound in this series, *p*-methoxyphenethyl alcohol (IX, Ar = *p*-methoxyphenyl). To our knowledge, no report on the occurrence of this compound has appeared.

Preliminary feeding experiments suggested that *p*-methoxyphenethyl alcohol could be expected to be a metabolite of *p*-methoxyphenylacetaldehyde oxime in those plants known to be capable of methylating phenolic hydroxyl groups. As *Aubrietia deltoidea* synthesizes glucoaubrietin (*p*-methoxybenzylglucosinolate VI, Ar = *p*-methoxyphenyl),[18] this organism was chosen to investigate the biosynthesis of *p*-methoxyphenethyl alcohol.

A typical infusion experiment with ^{14}C-labelled oxime was performed as follows: a solution of 10 μc *p*-methoxyphenylacetaldehyde oxime-1-^{14}C in 15 ml water (containing 5% ethanol) was infused into the aerial part of 80 plants of *A. hybrida*. After a period of 60 hr the plant material was extracted and the compounds fractionated according to their solubility (see Experimental). The hydrophilic compounds in the aqueous phase were separated by paper chromatography using system A. The main peak, R_f value 0·74–0·82, coincides with *S*-(β-D-glucopyranosyl)-*p*-methoxyphenylacetothiohydroxamic acid, a C_6-C_2 compound which is expected to be formed from the L-*p*-methoxyphenylalanine or *p*-methoxyphenylacetaldehyde oxime. But when 80% of the radioactive zone were cut out, eluted, and subjected to acid hydrolysis the chromatogram developed in system B revealed that only a very low percentage of the original activity was localized in the zone corres-

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ponding to *p*-methoxyphenylacetic acid ($R_f = 0{\cdot}60$). The chief amount of radioactivity (140,000 dis/min) appeared at a R_f value of 0·76. Rechromatography of this peak in solvent system A indicated that the compound after hydrolysis was not identical with that corresponding to the original spot on the first chromatogram. The mobility of the new compound was compared by TLC with a number of arylethane derivatives. Thus, it was assumed that the unknown material may be *p*-methoxyphenethyl alcohol. The radioactive material (120,000 dis/min) was diluted with 200 mg inactive *p*-methoxyphenethyl alcohol as carrier and distilled at 150° and 10^{-1} torr. A colourless oil was obtained with a specific activity of 98 dis/min/μmole. This alcohol was further converted into the *p*-nitrobenzoyl derivative (89 dis/min/μmole). When recrystallized three times from ethanol–light petrol the crystals had the following specific acitivities (dis/min/μmole): 83, 86 and 85. Also after fractional sublimation at 10^{-3} torr and 120° the specific activity remained constant (87 dis/min/μmole).

The structure of the original compound was established as the β-D-glucoside of *p*-methoxyphenethyl alcohol, because formation of the radioactive *p*-methoxyphenethyl alcohol was demonstrated only when the glucoside fraction was incubated with β-glucosidase but not with α-glucosidase. The aglycones which were obtained from the ether phase of the original partition were separated on TLC of silica gel. Further identification of *p*-methoxyphenethyl alcohol was carried out in the same way as described above.

In experiments using *p*-hydroxyphenylacetaldehyde oxime-1-^{14}C no transformation of this aldoxime into the *O*-methyl-derivatives of tyrosol could be detected, indicating that methylation takes place at the level of L-tyrosine. This is in accordance with the suggestion that the carbon skeleton of the side chain of glucosinolates is preformed prior to the *N*-hydroxylation leading to the aldoximes.

Results regarding the biosynthesis of *p*-methoxyphenethyl alcohol and its β-D-glucoside are summarized in Table 1. It was found that only small amounts of L-*p*-methoxyphenylalanine were incorporated into the tyrosol derivatives, whereas *p*-methoxyphenylacetaldehyde oxime was a good precursor. The findings are consistent with the view that the aldoxime is a more immediate precursor than the amino acid.

TABLE 1. INCORPORATION OF LABELLED PRECURSORS INTO *p*-METHOXYPHENETHYL ALCOHOL, ITS GLUCOSIDE AND *p*-METHOXYPHENYLACETALDEHYDE OXIME IN THE AERIAL PART OF *Aubrietia hybrida*

Compound fed*	Activity (μc)	Spec. act. (mc/mMol)	% Incorporation into: *p*-Methoxyphenethyl alcohol	% Incorporation into: Glucoside	% Incorporation into: Aldoxime
L-*p*-Methoxyphenylalanine-*O*-methyl-^{14}C	45	0·10	0·02	0·02	0·003
p-Methoxyphenylacetaldehyde oxime-1-^{14}C	10	0·054	0·5	0·8	—

* Over 60 hr into 150 g fr. wt. of *Aubretia*.

Biosynthesis of Tyrosol and its 1-β-D-*Glucoside*

While the application of L-tyrosine to *S. alba* or various species of *Aubrietia* led predominantly to the formation of known compounds such as homogentisic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid or sinalbin (*p*-hydroxyphenylglucosinolate VI, Ar = *p*-hydroxyphenyl), *p*-hydroxyphenylacetaldehyde oxime (the corresponding oxime of the

next lower aldehyde), although known as a plant constituent and metabolite of L-tyrosine, has a much more limited metabolism. One way leads to the nitrile, a precursor of the cyanogenic glucosides dhurrhin and taxiphyllin (IV, Ar = *p*-hydroxyphenyl), another way to sinalbin; but after feeding of *p*-hydroxyphenylacetaldehyde oxime-1-^{14}C, the main peak of radioactivity, apart from that at the origin of the chromatogram of the glycoside fraction, corresponds to a compound so far unknown in the Cruciferae. When the chromatographically purified substance was subjected to hydrolysis in HCl the radioactivity was localized in polymeric products and 2 phenolic compounds; one was recognized as tyrosol. Tyrosol, purified by repeated chromatography, was finally characterized by recrystallization to constant specific activity. After dilution with carrier, and recrystallization from benzene, the crystalline material exhibited the following specific acitivities (dis/min/μmole): 358, 362 and 360. After sublimation the specific activity was not changed. The second phenol was also obtained when an authentic sample of tyrosol was refluxed with HCl. It was identified as *p*-hydroxyphenethylchloride by NMR and mass spectrometry. The chloride and the resinous material were obviously secondary products of the HCl treatment of the glucoside.

In order to determine the structure of this glycoside, the radioactive substance was treated with dimethyl sulfate in NaOH. Hydrolysis of the methylated compound followed by chromatography gave a single radioactive zone on TLC chromatograms. The new compound showed no reaction with diazotized sulfanilic acid and was identical with *p*-methoxyphenethyl alcohol. Furthermore, the original glycosidic compound was hydrolyzed with purified β-glucosidase from almonds and showed no reaction with α-glucosidase from yeast. Thus, the tyrosol derivative resulting from *p*-hydroxyphenylacetaldehyde oxime must be 1-β-D-glucoside, 1(β-D-glucopyranosyl)-2(*p*-hydroxyphenyl) ethane.

The glucoside is further characterized by the R_x value of 1·4 in acetone–water (9:1, v/v) (related to glycerol) or R_f = 0·2 in solvent system I.

In order to elucidate the biosynthetic pathway to tyrosol, L-tyrosine-u-^{14}C and *p*-hydroxyphenylacetaldehyde oxime-1-^{14}C were fed to leaves of *S. alba* and various species

TABLE 2. COMPARISON OF LABELLED COMPOUNDS AS PRECURSORS OF TYROSOL, ITS GLUCOSIDE, *S*-(β-D-GLUCOPYRANOSYL)-*p*-HYDROXYPHENYLACETOTHIOHYDROXAMIC ACID AND *p*-HYDROXYPHENYLACETALDEHY DE OXIME

Exp. No.	Compounds fed	Activity (μc)	Spec. act. (mc/mMol)	Plant	Time (hr)	% Incorporation in: Tyrosol	Tyrosol glucoside	Thiohydroxamic acid	Aldoxime
1	L-Tyrosine-u-^{14}C	100	468	*A. graeca*	50	0·004	0·001	0·1	0·04
2	L-Tyrosine-u-^{14}C	100	468	*S. alba*	30	n.d.	0·002	0 1	n.d.
3	*p*-Hydroxyphenylacetaldehyde oxime-1-^{14}C	5·55	0·326	*S. alba*	50	0·4	1·8	1·3	—
4	*p*-Hydroxyphenylacetaldehyde oxime-1-^{14}C	1·3	0·0252	*S. alba*	50	n.d.	15·2	0 4	—
5	*p*-Hydroxyphenylacetaldehyde oxime-1-^{14}C	3·2	0·032	*S. alba*	50	n.d.	14·7	0 5	—
6	*p*-Hydroxyphenylacetaldehyde oxime-1-^{14}C	3·2	0·032	*A hendersonii*	80	n.d.	2 43	0·3	—

n.d.: Not determined.

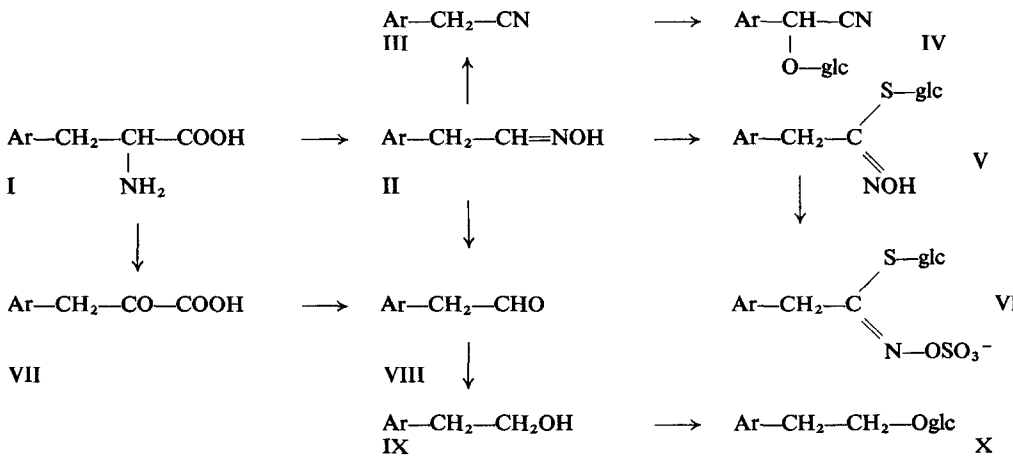
of *Aubrietia*. The two genera used in these experiments were chosen for different reasons. *S. alba* was selected because it synthesizes mustard oil glucosides and converts L-tyrosine to *p*-hydroxyphenylacetaldehyde oxime.[6] *Aubrietia* synthesizes *p*-methoxyaryl derivatives and is useful for studies where methylation of phenols is involved. As outlined in Table 2, the results show enough difference of incorporation into tyrosol, its β-D-glucoside and *S*-(β-D-glucopyranosyl)-*p*-hydroxyphenylacetothiohydroxamic acid (VI) to suggest that formation of tyrosol from L-tyrosine proceeds *via* aldoxime.

Phenylacetaldehyde Oximes as Metabolites of C_6C_3-Amino Acids

To prove that the aldoximes are not only precursors of the tyrosol derivatives but also intermediates in this biosynthetic pathway, the formation of the aldoximes as metabolites of the next higher amino acid had to be demonstrated. Although apparently only a low stationary concentration of the aldoximes is maintained in these plants, we were able to determine the formation of the two aldoximes after administration of the corresponding next higher amino acid (Tables 1 and 2). After repeated chromatography of the ether phase on silica gel (employing solvent system I and system II) the zones corresponding to the aldoximes were extracted with methanol and the eluate evaporated. In the case of Experiment 1 (Table 2) the radioactivity found in this eluate was 95,000 dis/min. After addition of 150 mg of highly purified authentic material the specific activity was 95 dis/min/μmole. This value was not altered very much by recrystallization from water; the third recrystallization gave a product with a specific activity of 84 dis/min/μmole. The aldoxime was finally converted into the nitrile (see Experimental), the latter was purified by preparative TLC and recrystallized from benzene–light petrol. The product obtained by this way showed no depression in melting point when admixed with an authentic sample and had a specific activity of 82 dis/min/μmole. An analogous procedure was employed in order to examine the formation of labelled *p*-methoxyphenylacetaldehyde oxime.

DISCUSSION

During this investigation it was established unequivocally that some Cruciferae can utilize aldoximes for the biosynthesis of alcohols such as tyrosol. A biosynthetic pathway from L-tyrosine to tyrosol derivatives consistent with the results of the tracer experiments, is outlined in Scheme 1.



SCHEME 1. BIOSYNTHESIS AND METABOLISM OF AROMATIC ALDOXIMES IN HIGHER PLANTS.

The free phenylacetaldehydes are here shown as hypothetical stages of the postulated reaction sequence, which may involve a transoximation step—a reaction already described in various organisms[19,20]—or, more likely, may proceed by hydrolysis. Evidence provided in support of the metabolism of L-amino acids to the next lower alcohol in the case of L-tyrosine also suggests that this type of reaction may play a more general role, at least in higher plants. Preliminary experiments concerning the metabolism of indolyl-3-acetaldehyde oxime which is formed from L-tryptophan,[21] are consistent with the hypothesis that such a pathway is also operative in the biosynthesis of tryptophol.[22] Since aromatic aldehydes like indolyl-3-acetaldehyde are oxidized to the corresponding acids, arylacetaldehyde oximes may be obligatory precursors of arylacetic acid in certain plants whereby either nitriles or aldehydes can function as intermediates.

In plants too, aromatic amino acids undergo reversible transamination, and *p*-hydroxyphenylacetaldehyde may be formed from *p*-hydroxyphenylpyruvic acid; experiments with plants of *S. alba* are consistent with the assumption that *p*-hydroxyphenylpyruvic acid is metabolized towards homogentisic acid and that only extremely low amounts of *p*-hydroxyphenylacetic acid are formed. Although a pathway L-tyrosine—α-ketoacid—next lower aldehyde cannot be excluded on the basis of these experiments, it remains the fact that transformations of amino acids to oximes of the next lower aldehydes and conversions of aldoximes into alcohols are operative. Hence we are in favour of the idea that in the higher plants investigated tyrosol primarily arises from *p*-hydroxyphenylacetaldehyde oxime. This is in contrast to the results obtained with microorganisms.[16,17] It would certainly be interesting to examine which pathway is predominantly contributing to the biosynthesis of tyrosol at different stages of development; but this question needs further experiments.

It is remarkable that the formation of glucosides of thiohydroxamic acids isolated from the experimental plants was markedly lower than the biosynthesis of tyrosol derivatives. The ratio of the formation of thiohydroxamic acid derivatives to the biosynthesis of tyrosol derivatives seems to depend on the amount of the aldoximes administered; material of high specific activity apparently brings about a preferential synthesis of the thiohydroxamic acid derivatives while the sequence leading to the alcohols dominates when unphysiologically large amounts of aldoximes are fed. This indicates that the high rate of incorporation is in part due to the unphysiological conditions of the feeding experiments. As there are higher plants which contain phenylethyl alcohol derivatives but which do not seem to form mustard oil glucosides or cyanogenic glucosides, it would be of interest to know whether the reaction sequence reported here also operates in this type of plant. If so, the biosynthesis of tyrosol and the pathway leading to the nitrogen containing glycosides would be entirely independent. Another question open to discussion is whether yeasts or even procaryotic organisms are able to form aldoximes like *p*-hydroxyphenylacetaldehyde oxime. As they are found to be capable of synthesizing *N*-hydroxy compounds (e.g. *N*-hydroxy peptides) it is tempting to speculate how widely *N*-hydroxylation is distributed.

EXPERIMENTAL

α-Glucosidase, type I, from yeasts (1 mg hydrolyses 3·0 μMol *p*-nitrophenyl-α-D-glucoside/min at pH 6·8, 37°), β-glucosidase from almonds (1 mg liberates 4·8 μMol D-glucose/min from salicin at pH 5·3, 37°C) and the α-glucoside and β-glucoside of *p*-nitrophenol were purchased from Sigma. Chemicals

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[22] H. KINDL, unpublished.

for comparison and radioactive compounds administered to the plants were obtained from commercial sources wherever possible.

TLC. Chromatograms were developed with the following systems: System I: Benzene–EtOAc = 4:1 (v/v); System II: CH_2Cl_2; System III: CH_2Cl_2–Et_2O = 10:1 (v/v).

Compound	R_f in System I R=H	R=CH_3	R_f in System II R=H	R=CH_3	R_f in System III R=H	R=CH_3
RO–C$_6$H$_4$–CH_2–CH_2–OH	0·15	0·39	0–0·025	0·19	0·125	0·44
RO–C$_6$H$_4$–CH_2–CH=NOH	0·27	0·50	0–0·03	0·13	0·17	0·45
RO–C$_6$H$_4$–CH_2–CHO	0·47	0·75	0·15	0·72	0·53	0 89
RO–C$_6$H$_4$–CH_2CN	0·57	0·82–0·9	0·18	0·67–0·75	0·60	0 93–1·0
RO–C$_6$H$_4$–CH_2CH_2–Cl	0·71	—		—		—
RO–C$_6$H$_4$–CH_2–CH_2–$OCOCH_3$	0·50	—		—		—

For preparative TLC PSC-Fertigplatten (Kieselgel F_{254} 20 × 20 cm, 2 mm thick, from Merck) were used, or plates with 0·8 mm thick silica gel layers were prepared. Silufol (Serva, Heidelberg) was employed for analytical work.

Paper chromatography. Mainly, the following developing solvents have been utilized: (*A*), acetone–benzene–H_2O, 18:1:1 (by vol.); (*B*), n-BuOH–conc. NH_4OH–EtOH–benzene, 5:3:2:1 (by vol.);[23,24] (*C*), benzene–HOAc–H_2O, 4:2:1 (by vol.).[23,24]

MN 218 (Machery and Nagel) and other thick papers were utilized for preparative separations.[25] Compounds were detected by spraying with fluorescence indicators (euchtstoff Grün, Rhodamin G), glucosides by developing with Ag^+.[25] Phenolics were visualized with diazotized sulfanilic acid in carbonate solution.[23]

The preparation of *p*-methoxyphenethyl alcohol, tyrosol, *p*-methoxyphenylacetaldehyde oxime-1-^{14}C and *p*-hydroxyphenylacetaldehyde oxime-1-^{14}C are described elsewhere.[6,26] L-*p*-Methoxyphenylalanine-*O*-methyl-^{14}C was synthesized with Me_2SO_4-^{14}C followed by hydrolysis. Purification and separation from the *N*-methyl derivatives was achieved by paper chromatography in solvent system B (R_f: L-tyrosine: 0·30, L-*p*-methoxyphenylalanine: 0·42).

1(p-*Nitrobenzoyloxy*)-2(p'-*methoxyphenyl*)-*ethane*

152 mg *p*-methoxyphenethyl alcohol were dissolved in 0·3 ml dried pyridine and 240 mg *p*-nitrobenzoylchloride in 3·0 ml pyridine added and the mixture allowed to stand 3 hr at 35°. The solvent was evaporated at 10 torr, the residue dissolved in $CHCl_3$ (10 ml), and unchanged acid extracted into saturated $NaHCO_3$. The $CHCl_3$ layer was washed, (H_2O), dried (Na_2SO_4), and concentrated. The ester was recrystallized from EtOH yielding yellow needles; m.p. 110°. R_f value on silica gel plates using benzene–EtOAc (15:1) as solvent: 0·50. (Calc. for $C_{16}H_{15}NO_5$: 63·8 C 5·00 H 4·65 N. Found: 63·9 C 4·95 H 4·66 N%.)

Conversion of *p*-methoxyphenylacetaldehyde oxime and *p*-hydroxyphenylacetaldehyde oxime into the corresponding nitriles: 5–20 mg oxime were dissolved in 2·0 ml dried benzene and, maintaining 70°, a solution of 500 μl $SOCl_2$ in 2·0 ml benzene was added dropwise over a period of 5 min. The mixture was refluxed very gently for 10 min and then the benzene distilled off *in vacuo*. The residue was applied on a TLC plate (2 mm thick) and purified using System I as developing solvent.

Administration of Labelled Compounds

Plants were grown under artificial light and controlled temperature. The various species of *Aubrietia* were more than 10-weeks-old while *S. alba* was usually 6-weeks-old. Labelled compounds were administered to leaves (*S. alba*) or to the aerial portion of the plants (*Aubrietia*). Precautions were taken to eliminate the formation of air blocks in the stem. Infusions were performed, employing V-shape glass beakers. A continuous light of lamps (Philips, TL 32 and TL 55) was applied during the infusion period.

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Isolation of Metabolites

Immediately after terminating the administration of labelled precursors the plant material was extracted by refluxing in hot MeOH for 5 hr (3×). 5 mg of each compound expected were added to the combined extracts which were then evaporated to dryness. The residue was shaken with a mixture of 50 ml Et_2O and 30 ml H_2O. The H_2O layer was then washed twice with Et_2O and the combined ether phase concentrated. This fraction containing the non-glycosidic phenols was further purified by preparative TLC using System I as solvent. The interesting zones were rechromatographed in System II and System I The identification of oximes (after conversion into nitriles and followed by TLC in System I and System II), tyrosol (by distillation and recrystallization to constant specific activity), and *p*-methoxyphenethyl alcohol (after acylation and recrystallization of the *p*-nitrobenzoyl derivative obtained) was accomplished by additional experiments.

The hydrophilic compounds of the aqueous layer were separated using preparative paper chromatography and System A for development. After rechromatography in System A or B these radioactive peaks were subjected to hydrolysis (by acids or glucosidases) and then characterized as aglycones by TLC

Radioactivity was assayed as described previously.[6,24]

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