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L-2-AMINO-4-PHENYLBUTYRIC ACID AND 2-PHENYLETHYLGLUCOSINOLATE, PRECURSORS OF 2-HYDROXY-2-PHENYLETHYLGLUCOSINOLATE*

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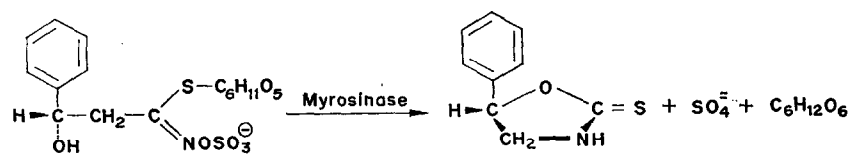
Abstract—The biosynthesis of (*S*)2-hydroxy-2-phenylethylglucosinolate was studied in *Reseda luteola* L. Labeled compounds (^{14}C and ^{15}N) were administered to plant shoots and the incorporation of tracer into either the aglycone or glucosinolate was measured. L-2-Amino-4-phenylbutyric acid was more efficiently converted into (*S*)2-hydroxy-2-phenylethylglucosinolate than were the stereoisomers of 2-amino-4-hydroxy-4-phenylbutyric acid. The C-2 and amino nitrogen of these precursors were incorporated into the glucosinolate as a unit. The presence of 2-phenylethylglucosinolate in the plant was confirmed. Results of tracer studies supported the biosynthetic scheme: phenylalanine → → 3-benzylmalic acid → → 2-amino-4-phenylbutyric acid → → 2-phenylethylglucosinolate → 2-hydroxy-2-phenylethylglucosinolate.

INTRODUCTION

EARLIER investigations into the biosynthesis of (*S*)2-hydroxy-2-phenylethylglucosinolate (Scheme 1) in *Reseda luteola* L. demonstrated that the carbon skeleton of the aglycone moiety could be derived from DL-2-amino-4-phenylbutyric acid.[1] Feeding experiments gave evidence suggesting 2-amino-4-phenylbutyric acid could arise from phenylalanine and acetate by a chain extension mechanism,[1] a sequence since confirmed in the biosynthesis of 2-phenylethylglucosinolate in *Nasturtium officinale* R. Br.[2]



SCHEME 1. (*S*)2-HYDROXY-2-PHENYLETHYLGLUCOSINOLATE IN *R. luteola* AND ITS AGLYCONE, (*R*)5-PHENYL-2-OXAZOLIDINETHIONE.

These experiments, however, did not indicate the source of nitrogen for (*S*)2-hydroxy-2-phenylethylglucosinolate nor at what point in the biosynthetic sequence the hydroxyl was introduced. The results described in the present study clarify these aspects of the biosynthesis of (*S*)2-hydroxy-2-phenylethylglucosinolate.

RESULTS

Table 1 compares the incorporation of ^{14}C and ^{15}N from labeled precursors into the

* NRCC No. 12269.

[1] E. W. UNDERHILL, *Can. J. Biochem.* **43**, 189 (1965).

[2] E. W. UNDERHILL, *Can. J. Biochem.* **46**, 401 (1968).

aglycone of (*S*)2-hydroxy-2-phenylethylglucosinolate by *R. luteola*. The data presented are representative of several feeding experiments.

The intermediate precursor efficiency of [1,2-^{14}C]3-benzylmalic acid (I) between that of DL-[3-^{14}C]phenylalanine (II) and DL-[2-^{14}C–^{15}N]2-amino-4-phenylbutyric acid (III) is consistent with previous studies which employed isotope competition experiments.[1] These results appear to confirm the earlier suggestion that 2-amino-4-phenylbutyric acid arises in *R. luteola* from phenylalanine and acetate by a chain extension mechanism.[1]

TABLE 1. INCORPORATION OF ^{14}C AND ^{15}N FROM LABELED PRECURSORS INTO THE AGLYCONE OF (*S*)2-HYDROXY-2-PHENYLETHYLGLUCOSINOLATE

Compound fed*				(*R*)5-Phenyl-2-oxazolidinethione isolated		
No.	(μCi)	(μCi/mmol)	Atoms % excess ^{15}N	%^{14}C converted	Dilution‡ ^{14}C	Dilution‡ ^{15}N
(I) [1,2-^{14}C]3-Benzylmalic acid	12·96	223		7·46§	138§	
(II) DL-[3-^{14}C]Phenylalanine	15·06	323		6·07	201	
(III) DL-[2-^{14}C-^{15}N]2-Amino-4-phenylbutyric acid	3·29	98	30·85	14·1	109	288
(IV) L-[2-^{14}C-^{15}N]2-Amino-4-phenylbutyric acid	3·01	98	30·85	29·5	83	113
(V) [2,3-^{14}C-^{15}N]2-Amino-4-hydroxy-4-phenylbutyric acid (2*S*,4*S*)†	4·31	130	16·74	15·4	120	113
(VI) [2,3-^{14}C-^{15}N]2-Amino-4-hydroxy-4-phenylbutyric acid (racemic mixture) of (2*S*,4*S*) and (2*R*,4*R*)†	4·84	130	16·74	5·0	423	396
(VII) [2,3-^{14}C-^{15}N]2-Amino-4-hydroxy-4-phenylbutyric acid (2*S*,4*R*)†	4·74	130	16·74	1·48	970	780
(VIII) [2,3-^{14}C-^{15}N]2-Amino-4-hydroxy-4-phenylbutyric acid (racemic mixture) of (2*S*,4*R*) and (2*R*,4*S*)†	5·09	130	16·74	0·59	3290	2730

* Labeled compounds were fed to *R. luteola* (60–80 g fr. wt).
† Configurations listed have been assumed (see Results).
‡ Dilution = $\frac{\text{Specific activity or atoms \% excess } ^{15}\text{N of compound fed}}{\text{Specific activity or atoms \% excess } ^{15}\text{N of oxazolidinethione}}$.
§ Calculation assumes loss of the terminal carbon of [1,2-^{14}C]3-benzylmalic acid on conversion to the glucosinolate.

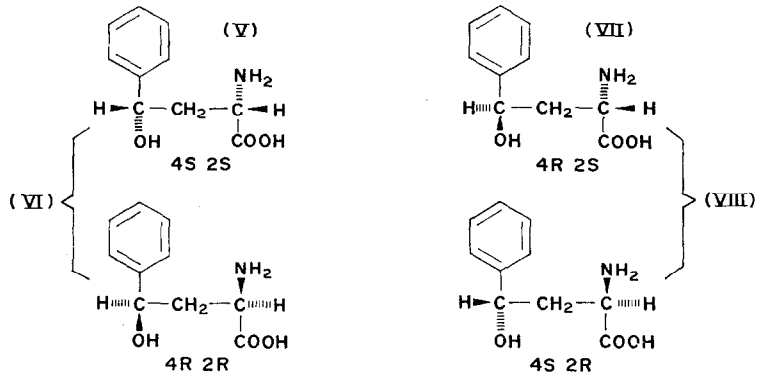
The most efficient precursor fed was L-[2-^{14}C–^{15}N]2-amino-4-phenylbutyric acid (IV). Repeated feeding experiments consistently demonstrated greater ^{14}C incorporation from (IV) than from stereoisomers of the amino acid structurally related to the glucosinolate, namely 2-amino-4-hydroxy-4-phenylbutyric acid (V–VIII).

The dissimilar dilution values for ^{14}C and ^{15}N on feeding doubly-labeled DL-2-amino-4-phenylbutyric acid (III) did not permit a conclusion regarding retention of the amino nitrogen. However, administration of the natural L-isomer (IV) showed incorporation of the C-2 and amino nitrogen as a unit into the glucosinolate, as is evident from similar dilution values for both the ^{14}C and ^{15}N. These results are consistent with the findings of a previous study on the biosynthesis of 2-phenylethylglucosinolate in *N. officinale*, from which it was concluded that the D-isomer underwent transamination to form the L-isomer prior to its conversion to the glucosinolate.

The four stereoisomers of doubly-labeled 2-amino-4-hydroxy-4-phenylbutyric acid were prepared and fed as represented in compounds (V)–(VIII). Whereas the stereochemistry of the individual compounds was not determined by direct experiment, configurations were deduced as follows. Since (V) was the most efficiently converted of the stereoisomers fed, we have assumed that (V) (Scheme 2) has the same stereochemistry about C-4 as that about C-2 of the glucosinolate, namely (*S*),[3–5] and therefore can be assigned as (2*S*,4*S*)2-amino-4-hydroxy-4-phenylbutyric acid. On the basis of this assumption, the stereochemistry of (VI),

[3] A. KJAER and R. GMELIN, *Acta Chem. Scand.* **11**, 906 (1957).
[4] A. KJAER and R. GMELIN, *Acta Chem. Scand.* **12**, 1693 (1958).
[5] R. GMELIN, A. KJAER and A. SCHUSTER, *Acta Chem. Scand.* **24**, 3031 (1970).

(VII) and (VIII) were assigned. Synthesis of 2-amino-4-hydroxy-4-phenylbutyric acid (see Experimental) afforded the diastereoisomeric pairs (VI) and (VIII) which were separated by chromatography according to the system used for separating *allo*phenylserine from phenylserine.[6] Since (V) (2*S*,4*S*) was obtained by treating racemic (VI) with a D-amino acid oxidase, then (VI) must be a mixture of (2*S*,4*S*) and (2*R*,4*R*). Racemic (VIII) then would be (2*S*,4*R*) and (2*R*,4*S*), which by action of D-amino acid oxidase would afford (VII) (2*S*,4*R*).



SCHEME 2. DIASTERIOISOMERS OF 2-AMINO-4-HYDROXY-4-PHENYLBUTYRIC ACID.

Retention of the amino nitrogen on feeding the DL-racemate of an amino acid has not been evident in previous studies[1,7–9] on the biosynthesis of glucosinolates. The novel demonstration of such retention obtained on feeding racemic (VI) can be explained on consideration of the stereochemistry of this DL-racemate. It is conceivable that on feeding (VI) neither the carbon skeleton nor the amino nitrogen of the (2*R*,4*R*) isomer was incorporated due to the 'inappropriate' configurations about both C-2 and C-4, whereas the ^{14}C of the (2*S*,4*S*) enantiomer was converted without loss of the nitrogen in like manner to (V).

Several recrystallizations were required to obtain a constant specific activity of isolated 5-phenyl-2-oxazolidinethione after feeding (VII) or (VIII), in contrast to the unchanged specific activity of the aglycone after the first recrystallization when either (V) or (VI) were fed. In each instance on feeding (VII) or (VIII), the initial specific activity was high, then decreased to a constant lower value on subsequent recrystallizations. On the basis of stereochemical considerations, compound (VII) would be expected to be a precursor not of the naturally occurring glucoside in *R. luteola*, but rather of its epimer. It is noteworthy that (*S*)5-phenyl-2-oxazolidinethione has been recently isolated from enzymatically hydrolysed extracts of *Sibara virginica* L.,[5] derived most likely from naturally occurring (*R*)2-hydroxy-2-phenylethylglucosinolate, an epimer of the predominant glucosinolate in *R. luteola*. It is possible that on feeding the ^{14}C, ^{15}N-labeled (2*S*,4*R*) amino acid, either alone (VII) or in the racemate (VIII), the label was incorporated into the (*R*) glucosinolate affording (*S*)5-phenyl-2-oxazolidinethione on hydrolysis, which was then at least partially removed by recrystallization from a larger amount of the (*R*) enantiomer arising from the (*S*) glucosinolate, predominant in *R. luteola*. This could be expected, in fact, since Gmelin *et al.*[5] report (*S*)5-phenyl-2-oxazolidinethione isolated from *S. virginica* extracts was partially racemic.

[6] K. N. F. SHAW and S. W. FOX, *J. Am. Chem. Soc.* **75**, 3421 (1953).
[7] M. MATSUO and M. YAMAZAKI, *Biochem. Biophys. Res. Commun.* **25**, 269 (1966).
[8] C. LEE and G. SERIF, *Biochemistry* **9**, 2068 (1970).
[9] E. W. UNDERHILL, *Can. J. Biochem.* **43**, 179 (1965).

The incorporation of tracer from compounds (I), (IV), (V) and (VII) into the glucosinolate was specific. Degradation of resulting 5-phenyl-2-oxazolidinethione revealed the thione carbon contained 88–94% of the theoretical activity required for specific incorporation of these precursors.

The presence of 2-phenylethyl isothiocyanate in the steam distillate of *R. luteola* extracts has been reported on the basis of paper chromatography.[10] We have confirmed the occurrence of this isothiocyanate in *R. luteola* and have identified its parent glucosinolate. GLC analysis, on two different phases, of the steam distillate of a myrosinase treated extract of the plant showed a component with the same relative retention time (RRT) as that of authentic 2-phenylethyl isothiocyanate. When the glucosinolate fraction from *R. luteola* was chromatographed on DE-32,[11] two glucosinolates, one as a shoulder on the major peak, were detected (UV at 254 nm) in the column eluate. Trimethylsilylation of the mixture and subsequent GLC analysis on SE-52 and OV-225 columns[12] confirmed the presence of a minor glucosinolate with retention times corresponding to those of the TMS derivative of authentic 2-phenylethylglucosinolate.

Table 2 presents data obtained from one of a number of feeding experiments in which DL-[2-^{14}C]2-amino-4-phenylbutyric acid was administered to *R. luteola* shoots and the specific activities of the aglycone moieties of both 2-phenylethylglucosinolate and 2-hydroxy-2-phenylethylglucosinolate were determined. In each instance, the specific activity of 2-phenylethyl isothiocyanate was greater than that of 5-phenyl-2-oxazolidinethione, a result which suggests that 2-phenylethylglucosinolate could be an intermediate in the biosynthesis of 2-hydroxy-2-phenylethylglucosinolate.

TABLE 2. INCORPORATION OF ^{14}C FROM DL-[2-^{14}C]2-AMINO-4-PHENYLBUTYRIC ACID INTO THE AGLYCONES OF 2-PHENYLETHYLGLUCOSINOLATE AND (*S*)2-HYDROXY-2-PHENYLETHYLGLUCOSINOLATE

2-Phenylethyl isothiocyanate			(*R*)5-Phenyl-2-oxazolidinethione			Plant glucosinolate dilution†
(μCi/mmol)	% ^{14}C converted	Dilution	(μCi/mmol)	% ^{14}C converted	Dilution	
3·32	2·14	30	0·938	26·9	104	4
4·31	4·08	23	0·781	24·0	125	6
3·76	5·95	26	0·791	16·9	123	5

* Labeled DL-[2-^{14}C]2-amino-4-phenylbutyric acid (98 μCi mmol) were fed to *R. luteola* (60–70 g fr. wt). 1·65, 1·57 and 1·67 μC were fed respectively in the three experiments.

† Plant glucosinolate dilution $= \frac{\text{Specific activity of 2-phenylethyl isothiocyanate}}{\text{Specific activity of 5-phenyl-2-oxazolidinethione}}$.

Efficient conversion of the desoxy glucosinolate into 2-hydroxy-2-phenylethylglucosinolate is further suggested by low 'plant glucosinolate dilution' values, obtained by dividing the specific activity of isolated 2-phenylethylglucosinolate by that of 2-hydroxy-2-phenylethylglucosinolate or their aglycones. The greater incorporation of ^{14}C into the oxazolidinethione is reasonable in view of the relatively large natural pool size of the parent 2-hydroxy-2-phenylethylglucosinolate, approximately 25 times that of 2-phenylethylglucosinolate.

[10] P. DELAVEAU, *Bull. Soc. Bot. Fr.* **104**, 148 (1957).
[11] E. W. UNDERHILL and L. R. WETTER, *Plant Physiol.* **44**, 584 (1969).
[12] E. W. UNDERHILL and D. F. KIRKLAND, *J. Chromatogr.* **57**, 47 (1971).

To determine whether, in fact, 2-phenylethylglucosinolate could be converted in the plant to its hydroxylated analogue, [G-^{14}C]2-phenylethylglucosinolate was fed to *R. luteola*. Both glucosinolates were then isolated, purified (GLC) as their TMS derivatives, and the specific activities determined. The dilution values obtained, presented in Table 3, clearly indicate the efficient conversion of [G-^{14}C]2-phenylethylglucosinolate into its hydroxylated analogue. It is significant that the same 'plant glucosinolate dilution' values were obtained from feeding either DL-[3-^{14}C]phenylalanine or [G-^{14}C]2-phenylethylglucosinolate. Furthermore, these values were the same as those found (Table 2) on feeding DL-[2-^{14}C]2-amino-4-phenylbutyric acid.

TABLE 3. INCORPORATION OF ^{14}C INTO 2-PHENYLETHYLGLUCOSINOLATE AND (*S*)2-HYDROXY-2-PHENYLETHYLGLUCOSINOLATE

Compound fed*		2-Phenylethylglucosinolate		(*S*)2-Hydroxy-2-phenylethylglucosinolate		Plant glucosinolate dilution†
	(μCi)	(μCi/mmol)	Dilution	(μCi/mmol)	Dilution	
DL-[3-^{14}C]Phenylalanine	13·05	264	4·91	54	1·77	149	3
[G-^{14}C]2-Phenylethylglucosinolate	2·71	64	13·8	5	2·35	27	6
	2·74	64	10·6	6	2·84	23	4

* Labeled compounds were fed to *R. luteola* (60–75 g fr. wt).

† Plant glucosinolate dilution = $\frac{\text{Specific activity of 2-phenylethylglucosinolate isolated}}{\text{Specific activity of 2-hydroxy-2-phenylethylglucosinolate}}$.

DISCUSSION

It is apparent from these results that *R. luteola* can efficiently convert both L-2-amino-4-phenylbutyric acid (IV) and (2*S*,4*S*)2-amino-4-hydroxy-4-phenylbutyric acid (V) into the hydroxylated glucosinolate with the amino nitrogen intact. Consistently greater incorporation of (IV), however, argues against this amino acid being hydroxylated and converted via (V) into the glucosinolate. Furthermore, the relative specific activities of the two glucosinolates isolated on feeding (IV) (Table 2), strongly suggest incorporation of (IV) via 2-phenylethylglucosinolate and hydroxylation of this glucosinolate as the final step in the biosynthesis of 2-hydroxy-2-phenylethylglucosinolate. The high efficiency of conversion of tracer on feeding labeled 2-phenylethylglucosinolate provides additional data in support of the hydroxylated glucosinolate arising from its desoxy analogue. Further, the consistency of the 'plant glucosinolate dilution' values obtained when phenylalanine, 2-amino-4-phenylbutyric acid, and 2-phenylethylglucosinolate were fed provides evidence for a single biosynthetic pathway involving these compounds. We propose that the naturally functioning pathway is phenylalanine → → 2-amino-4-phenylbutyric acid → → 2-phenylethylglucosinolate → 2-hydroxy-2-phenylethylglucosinolate, otherwise the relative specific activities of the two isolated glucosinolates (i.e. the 'plant glucosinolate dilution' values) could be expected to differ in magnitude according to the pathway followed by the precursor fed.

The route by which (2*S*,4*S*)2-amino-4-hydroxy-4-phenylbutyric acid (V) was incorporated into the (*R*)5-phenyl-2-oxazolidinethione aglycone is open to question. However, formation of the enantiomeric (*S*) oxazolidinethione on feeding (VII) (see Results) necessitates retention of the hydroxyl of 2-amino-4-hydroxy-4-phenylbutyric acid throughout its conversion

to the glucosinolate and suggests that (V) and (VII) may have been converted into the epimeric glucosinolates as a result of low substrate specificity of the enzymes involved.

Our conclusion that hydroxylation of 2-phenylethylglucosinolate is the final step in the biosynthesis of 2-hydroxy-2-phenylethylglucosinolate supports the recent findings of Lee and Serif[13] which indicate hydroxylation in the biosynthesis of 2-hydroxy-3-butenylglucosinolate occurs at a point beyond formation of the intermediate aldoxime derived from its corresponding amino acid. The conversion of [G-^{14}C]-2-phenylethylglucosinolate into 2-hydroxy-2-phenylethylglucosinolate provides the first experimental confirmation of earlier suggestions[14,15] of a biogenetic relationship between hydroxy glucosinolates and their desoxy analogues. Additionally, the pathway proposed in this communication may actually represent a general biosynthetic pathway for the formation of all 2- and 3-hydroxy glucosinolates since, without exception,* the natural occurrence of the corresponding desoxy analogue is known.

EXPERIMENTAL

[2,3-^{14}C]Maleic anhydride, DL-[3-^{14}C]phenylalanine, [2-^{14}C]sodium acetate and $^{15}NH_4Cl$ were obtained from commercial sources; [1,2-^{14}C]3-benzylmalic acid[2] and both DL- and L-[2-^{14}C–^{15}N]2-amino-4-phenylbutyric acid[9] were prepared previously. [2,3-^{14}C–^{15}N]2-Amino-4-hydroxy-4-phenylbutyric acid was synthesized by the following sequence of reactions: [2,3-^{14}C]maleic anhydride (0·5 mmole, 200 μCi, purified by sublimation) and $AlCl_3$ (1·04 mmol) were taken in 1·5 ml of benzene and the mixture refluxed for 1 hr with stirring. After cooling the mixture on ice, 0·3 ml of H_2O and 0·075 ml of 12 N HCl were added and reflux continued for an additional 40 min. An Et_2O extract (2 × 1 ml) of the reaction mixture was extracted with 0·5 ml of 16% Na_2CO_3, the aqueous extract acidified with HCl, filtered, and the precipitate rinsed with cold H_2O. The dried product ([2,3-^{14}C]benzoylacrylic acid) in 2·5 ml of EtOH was cooled in a dry ice-EtOH bath and treated with $^{15}NH_3$ liberated from $^{15}NH_4Cl$ (1·06 mmol, 96·9 atom % excess ^{15}N) by 5 ml of 40% NaOH. The resulting intermediate [2,3-^{14}C–^{15}N]3-benzoylalanine was taken up in 15 ml H_2O, the EtOH removed, and the aqueous solution added dropwise to a stirred solution of $NaBH_4$ (1·6 mmol in 2 ml of H_2O). After stirring 16 hr, the mixture was acidified with 10 N H_2SO_4, put onto an Amberlite IR-120 (H^+) column (1 × 12 cm), eluted with 1·5 N NH_4OH, and the eluate evaporated to dryness to give [2,3-^{14}C–^{15}N]2-amino-4-hydroxy-4-phenylbutyric acid (48·9 mg; m.p. 215–216°, recryst. from 50% EtOH). Paper chromatography (Whatman No 4, equilibrated overnight, BuOH–H_2O–acetone–NH_4OH, 8:6:1:1)[6] indicated two racemic mixtures (VI and VIII, R_f 0·45 and 0·57, respectively) which were separated on a Whatman cellulose column (2 × 50 cm) using the same solvent system. The racemates were resolved into their respective L-isomers (V and VII) by treatment with a D-amino acid oxidase.[9]

[G-^{14}C]2-Phenylethylglucosinolate was prepared by feeding $^{14}CO_2$ (3·5 mCi, 29 mCi/mmol) to *N. officinale* and isolated as the K salt.[11] The glucosinolate was reprecipitated from an EtOH–MeOH mixture to constant specific activity which did not change on GLC (OV-225) of the TMS derivative.[12] A single radioactive spot was detected on paper (BuOH–HOAc–H_2O, 4:1:1·8) and TLC (BuOH–PrOH–HOAc–H_2O, 3:1:1:1).

Labeled compounds were administered to *R. luteola* as previously described[1,11] and the shoots were allowed to metabolize for 24 hr under continuous light. (*R*)5-Phenyl-2-oxazolidinethione was isolated as previously described[1] and recrystallized from 25% EtOH to a constant specific activity (m.p. 124–125°). 2-Phenylethyl isothiocyanate (Table 2) was separated from the oxazolidinethione by steam distillation of the $CHCl_3$ extract of the myrosinase treated plant extract. 2-Phenylethyl isothiocyanate was recovered by Et_2O extraction of the NaCl-saturated distillate and purified by GLC using a 5 mm o.d. × 1·8 m column of 20% (w/w) FFAP on acid washed, DMCS-treated Chromosorb W, 60–80 mesh, at 220° and a 10:1 stream splitter. The isothiocyanate was collected in Et_2O cooled to −70°, then quantitatively assayed by the internal standard method using a 5 mm o.d. × 1·8 m column packed with 10% (w/w) SE-30 on DMCS-Chromosorb

* The series of desoxy glucosinolates has been recently completed with the isolation of isobutyl isothiocyanate formed upon myrosinase hydrolysis of 2-methylpropylglucosinolate in an extract of *Conringia orientalis* (L.) Andrz.[16]

[13] C. LEE and G. SERIF, *Biochim. Biophys. Acta* **230**, 462 (1971).
[14] A. KJAER and B. CHRISTENSEN, *Acta Chem. Scand.* **13**, 1575 (1953).
[15] M. G. ETTLINGER and A. KJAER, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), Vol. 1, p. 59, Appleton–Century–Crofts, New York (1968).
[16] E. W. UNDERHILL and D. F. KIRKLAND, *Phytochem.* **11**, 2085 (1972).

W (70–80) at 155° (RRT = 1·84, 2-phenylethyl isothiocyanate relative to methyl caproate = 1·00) before determination of the specific activity. (*R*)5-Phenyl-2-oxazolidinethione was recovered by $CHCl_3$ extraction of the residual solution in the steam distillation flask, and recrystallized to constant specific activity.

Isolation of 2-hydroxy-2-phenylethylglucosinolate and 2-phenylethylglucosinolate was carried out according to the method of Underhill and Wetter.[11] Column chromatography (DE-32) resulted in an overlap of the two glucosinolates which were then completely resolved by GLC of their TMS derivatives on 1·5% OV-225 (5 mm o.d. × 1·8 m).[12] The collected TMS derivatives were rinsed separately from the collection tubes with *n*-hexane, *n*-hexacosane internal standard was added, and the isolates were quantitatively assayed[12] for subsequent determination of specific activities.

2-Phenylethyl isothiocyanate in myrosinase treated glucosinolate extracts of *R. luteola* was initially confirmed by isolation of this aglycone as described above. The same retention times for the plant derived isothiocyanate and authentic 2-phenylethyl isothiocyanate relative to an internal standard were observed on GLC analysis of the $CHCl_3$ extract of the steam distillate. On 20% FFAP the RRT of 2-phenylethyl isothiocyanate was 0·67 (relative to methyl stearate = 1·00) and on 10% SE-30 the RRT of the isothiocyanate was 0·75 (relative to methyl laurate = 1·00). Determination of the distribution of activity in isolated (R)5-phenyl-2-oxazolidinethione has been described.[1] Analyses of ^{15}N and ^{14}C were carried out as previously described.[9,11]

Key Word Index—*Reseda luteola*; Resedaceae; glucosinolates: biosynthesis: 2-hydroxy-2-phenylethylglucosinolate.