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SHORT COMMUNICATION

A NEW THIOGLUCOSIDE, 2-METHYLPROPYLGLUCOSINOLATE

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Abstract—Based on relative efficiencies of conversion of ^{14}C from feeding labeled compounds to *Conringia orientalis* (L.) Andrz., the aglycone moiety of 2-hydroxy-2-methylpropylglucosinolate has been found to be formed from valine and the methyl carbon of acetate via leucine. Isobutyl isothiocyanate has been identified as the aglycone formed upon myrosinase hydrolysis of a new thioglucoside, 2-methylpropylglucosinolate, present in fresh plants of *C. orientalis*. It is suggested that 2-methylpropylglucosinolate may be an intermediate in the biosynthetic pathway between leucine and 2-hydroxy-2-methylpropylglucosinolate.

INTRODUCTION

LABELED precursor studies have demonstrated that a number of glucosinolate aglycones are derived in higher plants from commonly occurring amino acids or from their higher homologues formed by chain extension via acetate.[1] Lee and Serif have demonstrated the efficient conversion of labeled 2-amino-6-(methylthio)caproic acid[2] and 5-(methylthio)valeraldoxime[3] into 2-hydroxy-3-butenylglucosinolate (progoitrin) and have suggested that introduction of the hydroxyl group occurs at a later stage in the biosynthetic pathway. In a recent study on the biosynthesis of another 2-hydroxylated glucosinolate,[4] we have found that (*S*)2-hydroxy-2-phenylethylglucosinolate is formed in *Reseda luteola* L. from its desoxy analogue, 2-phenylethylglucosinolate. On the basis of structural similarities we have considered that the aglycone moiety of 2-hydroxy-2-methylpropylglucosinolate may be derived from leucine by a biosynthetic pathway similar to the formation of (*S*)2-hydroxy-2-phenylethylglucosinolate from 2-amino-4-phenylbutyric acid. Gmelin and Kjaer,[5] who have reported the occurrence of 2-hydroxy-2-methylpropylglucosinolate in *Reseda alba* L., have suggested a similar biogenetic pathway. The fact that the desoxy analogue of 2-hydroxy-2-methylpropylglucosinolate was unknown suggested that some differences in the formation of these two hydroxylated glucosinolates may be expected.

In this communication we report results of plant feeding experiments which confirm the precursor function of leucine. The detection of a new glucosinolate affording isobutyl isothiocyanate upon enzymic hydrolysis is also reported.

RESULTS AND DISCUSSION

Table 1 summarizes results obtained from one of a number of plant feeding experiments in which labeled compounds were administered to *C. orientalis* shoots. [G-^{14}C]D-Glucose

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[1] 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).
[2] C.-J. LEE and G. S. SERIF, *Biochem.* **9**, 2068 (1970).
[3] C.-J. LEE and G. S. SERIF, *Biochim. Biophys. Acta* **230**, 462 (1971).
[4] E. W. UNDERHILL and D. F. KIRKLAND, *Phytochem.* in press.
[5] R. GMELIN and A. KJAER, *Phytochem.* **9**, 599 (1970).

TABLE 1. COMPARISON OF ^{14}C-LABELED COMPOUNDS AS PRECURSORS OF THE AGLYCONE OF 2-HYDROXY-2-METHYLPROPYLGLUCOSINOLATE

Compound fed	μCi fed	Spec. act. μCi/mmol	Fresh wt. of shoot	5,5-Dimethyl-2-oxazolidinethione Spec. act. μCi/mmol	5,5-Dimethyl-2-oxazolidinethione %^{14}C converted*	5,5-Dimethyl-2-oxazolidinethione Dilution†
[G-^{14}C]D-Glucose	12·09	224	41	0·43	0·48	521
[G-^{14}C]L-Leucine	9·55	197	56	5·24	13·6‡	31‡
[G-^{14}C]L-Valine	8·64	148	47	1·11	2·0‡	106‡
[1-^{14}C]Sodium acetate	9·88	97	81	0·01	0·01	24 200
[2-^{14}C]Sodium acetate	6·65	73	72	0·53	2·0	138

* $\%^{14}C \text{ converted} = \frac{\text{specific activity of aglycone} \times \text{amount isolated}}{^{14}\text{C activity taken up by shoot}} \times 100.$

† $\text{Dilution} = \frac{\text{specific activity of compound fed}}{\text{specific activity of 5,5-dimethyl-2-oxazolidinethione.}}$

‡ Calculation assumes the loss of the carboxyl carbon of the precursor fed on conversion into the glucosinolate.

was fed to provide a measure of the relative efficiencies of the other administered compounds as precursors of the glucosinolate aglycone. [G-^{14}C]L-Leucine was the most efficient precursor with over 13% of its ^{14}C incorporated into 5,5-dimethyl-2-oxazolidinethione, the aglycone moiety of 2-hydroxy-2-methylpropylglucosinolate. The efficiency of conversion of tracer into the aglycone from [G-^{14}C]L-valine was similar to that from the methyl carbon of acetate and was intermediate between that from leucine and glucose. As expected,[1,6,7] there was only slight conversion of ^{14}C from the carboxyl of acetate. The results are entirely consistent with a biosynthetic pathway involving chain extension of valine by acetate, forming leucine,[8,9] and its subsequent conversion to the aglycone moiety of 2-hydroxy-2-methylpropylglucosinolate.

Apart from lacking evidence for intermediacy of the desoxy glucosinolate, the foregoing pathway is analogous to the chain extension of phenylalanine to 2-amino-4-phenylbutyric acid and its conversion via 2-phenylethylglucosinolate into 2-hydroxy-2-phenylethylglucosinolate.[4] Since the desoxy analogues of all other 2-hydroxy substituted glucosinolates, except 2-hydroxy-2-methylpropylglucosinolate, are known to occur in nature,[1] we were prompted to determine whether the occurrence of 2-methylpropylglucosinolate in *C. orientalis* could be established.

In our preliminary investigations we were unable to detect the presence of either 2-methylpropylglucosinolate or its isothiocyanate aglycone in *C. orientalis* seed extracts, a result confirming earlier work reported by Kjaer *et al.*[10] However, a hot methanolic extract of fresh plants bearing flowers and some immature fruit afforded an aqueous fraction which, in the presence of myrosinase, liberated 5,5-dimethyl-2-oxazolidinethione as the major product of enzymic hydrolysis and trace amounts of a steam volatile isothiocyanate. The steam volatile component, purified by GLC, exhibited the same retention times as those of authentic isobutyl isothiocyanate when chromatographed on three liquid phases, and gave

[6] M. D. CHISHOLM and L. R. WETTER, *Can. J. Biochem.* **42,** 1033 (1964).
[7] E. W. UNDERHILL, *Can. J. Biochem.* **46,** 401 (1968).
[8] M. STRASSMAN and L. N. CECI, *J. Biol. Chem.* **238,** 2445 (1962).
[9] G. W. BUTLER and L. SHEN, *Biochim. Biophys. Acta* **71,** 456 (1963).
[10] A. KJAER, R. GMELIN and R. BOE JENSEN, *Acta Chem. Scand.* **10,** 432 (1956).

IR and NMR spectra which were identical with those of isobutyl isothiocyanate. The amount of isothiocyanate isolated was estimated by GLC (internal standard method) to be 0·5 mg/kg fresh weight. The identity of the component was further substantiated by conversion of the isothiocyanate with ammonia into a thiourea derivative which exhibited the same paper and thin-layer chromatographic properties, UV and IR spectra as those of isobutylthiourea. On the basis of the structure of other progenitors of isothiocyanates formed from glucosinolates upon treatment with myrosinase,[1,11] the identity of the parent glucoside from which isobutyl isothiocyanate was derived would be 2-methylpropylglucosinolate. The appearance of this glucosinolate in *C. orientalis* provides support for the suggestion[4] that 2-hydroxylated glucosinolates are derived from their desoxy analogues. The limited natural abundance of 2-methylpropylglucosinolate in this plant has thwarted experimental testing of this suggestion.

EXPERIMENTAL

Precursor feeding experiments. Labeled compounds, obtained from commercial sources, were administered through the cut shoot of *C. orientalis* (L). Andrz. (bearing flowers and fruit) as described previously[12] and each plant was allowed to metabolize for 22 hr under continuous light. The shoot was cut into 4 vol. of boiling MeOH and after 10 min at reflux temperature was ground in a Waring Blendor. The ground material was re-extracted using 4 vol. of boiling 80% MeOH. The combined extract was concentrated to a small vol. and the aqueous mixture filtered through a pad of Celite analytical filter aid (Johns–Manville). The filtrate was treated with lead acetate solution to remove impurities and the excess lead precipitated as $PbPO_4$.[10] The pH was adjusted to 6·5 and the glucosinolate containing solution was extracted three times with equal vol. of $CHCl_3$; the $CHCl_3$ extract was discarded. A solution of myrosinase was added to the $CHCl_3$-extracted filtrate which was allowed to stand at room temp. overnight to permit complete cyclization of the liberated glucosinolate aglycone. 5,5-Dimethyl-2-oxazolidinethione was recovered by extraction into $CHCl_3$. The dried $CHCl_3$ extract was evaporated to dryness and the crude oxazolidinethione, dissolved in C_6H_6, was treated with decolorizing charcoal and then crystallized by addition of light petroleum. 5,5-Dimethyl-2-oxazolidinethione was recrystallized to constant specific activity from benzene–light petroleum and then from CCl_4. ^{14}C was assayed as described previously.[12]

Detection of isobutyl isothiocyanate. C. orientalis was collected from our experimental plots at the flowering stage of development. The entire plants (4 kg) were cut into boiling MeOH (16 l.) and extracted as described above. After removal of MeOH and filtration through Celite, the pH of the extract was adjusted to 6·5 and the mixture filtered again. The filtrate was extracted continuously for 48 hr with CH_2Cl_2 and the extract reserved (extract 'A'). A solution of myrosinase was added to the CH_2Cl_2-extracted aqueous fraction and, after standing 24 hr, was extracted continuously with CH_2Cl_2 for 40 hr (extract 'B'). Preliminary GLC analysis of concentrated (2–3 ml) extracts A and B on 20% FFAP (125°) and 10% UC-W98 (110°) columns (see below) indicated the presence of a component in extract 'B' but not in 'A' which had a retention time corresponding to that of isobutyl isothiocyanate. Extract B was steam distilled to separate 5,5-dimethyl-2-oxazolidinethione and other non-volatile components. The distillate (*ca.* 100 ml) was extracted continuously for 40 hr with CH_2Cl_2, the CH_2Cl_2 dried (Na_2SO_4) and concentrated to 0·3 ml. The steam volatile component with retention time corresponding to isobutyl isothiocyanate was purified by GLC on a Hewlett–Packard model 5754 instrument equipped with a 10:1 stream splitter. Injector and detector temperatures were maintained at 200°. Flow rates were 30 ml/min for He (carrier gas), 20 ml/min H_2 and 250 ml/min air supplied to the FID. Stainless steel columns (3 mm o.d. × 1·8 m) which were operated isothermally included 20% FFAP (Varian Aerograph) on Chromosorb W, acid-washed and DMCS treated, 60/70 mesh, 125°; 10% UC-W98 on Diatoport S (Hewlett–Packard), 80/100 mesh, 110°; and 6% OV-17 on Diatoport S, 80/100 mesh, 100°. Material was collected by condensation in solid CO_2 cooled glass tubes. The component, first recovered from the UC-W98 column, was rechromatographed and collected from the FFAP column.

A portion of the GLC-collected material was centrifuged into AgCl microcells (0·01 mm path length, Beckman 'Extrocells') and the IR spectrum recorded using a Perkin–Elmer 257 grating spectrophotometer equipped with a beam condenser. Isobutyl isothiocyanate, synthesized from isobutylamine and thiophosgene in the presence of base,[13] was similarly processed. The naturally derived and synthetic isothiocyanates exhibited coinciding spectra with strong IR bands at 695, 1295, 1345, 1390, 1445, 1468, 2090 2170, 2880, 2930 and 2970 cm^{-1}. Another portion of the collected material was taken into 50 μl of CH_2Cl_2 containing *n*-butyl

[11] A. KJAER, *Progr. Chem. Org. Nat. Prod.* **18,** 122 (1960).
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[13] A. KJAER, R. GMELIN and I. LARSEN, *Acta Chem. Scand.* **9,** 1143 (1955).

isothiocyanate and the relative retention times (RRTs) of the two components were compared with those obtained using a mixture of isobutyl isothiocyanate and *n*-butyl isothiocyanate. Identical RRTs for the isolated compound and for isobutyl isothiocyanate relative to that of *n*-butyl isothiocyanate (RRT = 1·00) were obtained, namely, 0·73, 0·71 and 0·76 when the mixtures were chromatographed isothermally on FFAP (125°), OV-17 (100°) and UC-W98 (110°), respectively. The remainder of the collected fraction was taken into CCl_4 and its NMR spectrum (Varian HA-100 spectrometer) showed signals at δ 1·02 [6H, doublet, J = 6·5 Hz, $(CH_3)_2$], δ 2·02 [1H, septet, J = 6·5 Hz, CH] and δ 3·32 [2H, doublet, J = 6·0 Hz, CH_2]. Most of the CCl_4 was removed by cautious evaporation and the residue was treated with a saturated solution of NH_3 in MeOH. The crude thiourea solution was concentrated (*ca.* 0·05 ml) and aliquots were chromatographed. Thiourea spots were detected on paper chromatograms with Grote's reagent[14] and on TLC by UV. The R_{Ph}* value obtained when chromatographed on Whatman No. 1 paper using H_2O-saturated $CHCl_3$[15] was 0·78, the same as that obtained using authentic isobutyl thiourea. The migration of the *C. orientalis* derived thiourea and synthetic isobutyl thiourea on Silica Gel F_{254} using the lower phase of $CHCl_3$–EtOAc–H_2O, 3:3:4[16] was the same, R_f 0·37. The UV spectrum in EtOH exhibited a maximum at 243 nm; the IR spectrum (KBr) was superimposable upon that of isobutylthiourea.

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[15] A. KJAER, J. CONTI and I. LARSEN, *Acta Chem. Scand.* **7,** 1276 (1953).
[16] H. WAGNER, L. HORHAMMER and H. NUFER, *Arzheimittel-Forsche.* **15,** 453 (1965).

* The R_{Ph}-value signifies the R_f value relative to that of phenylthiourea.

Key Word Index—Conringia orientalis; Cruciferae; glucosinolates; biosynthesis; 2-hydroxy-2-methylpropylglucosinolate.