ACTA CHEMICA SCANDINAVICA 17 (1963) 1515–1520

Glucolepigramin, a New Thioglucoside, Present in *Lepidium graminifolium* L. *

PALLE FRIIS and ANDERS KJÆR

Department of Organic Chemistry, Royal Veterinary and Agricultural College, Copenhagen, Denmark

According to paper chromatography, seeds of the crucifer *Lepidium graminifolium* L. contain two thioglucosides, one of which produces an orange colour with diazotized sulphanilic acid. Acid hydrolysis of the thioglucoside mixture produces two acids which can be separated by vapour phase chromatography of the corresponding methyl esters. Mass spectrometry indicates these to be the esters of a monohydroxyphenylacetic acid and the corresponding *O*-methyl derivative. On critical comparison with synthetic reference samples, the two esters are proved to be the formerly unknown methyl *m*-hydroxyphenylacetate and the corresponding *O*-methyl derivative.

Consequently, one of the thioglucosides is identical with glucolimnanthin, formerly found only in *Limnanthes Douglasii* R.Br. The second thioglucoside, which is proved to be of the usual structural type, (I), has not been previously found in Nature. The thioglucoside ion, for which the name *glucolepigramin* is proposed, is isomeric with that of the classical thioglucoside sinalbin.

The crucifer *Lepidium graminifolium* L. is a perennial herb, widely distributed throughout the Mediterranean region and extending into Middle Europe. In connexion with systematic studies of the distribution of isothiocyanate-producing glucosides in species of the genus *Lepidium*, it was observed in this laboratory several years ago [1] that the seed of *L. graminifolium* on paper chromatography exhibited a pattern of isothiocyanate-producing glucosides significantly different from that of most other *Lepidium* species, containing glucotropaeolin as the major thioglucoside and consequently producing benzyl isothiocyanate on enzymic hydrolysis. The only published report on the presence of mustard oil glucosides in *L. graminifolium* is that of Delaveau,[2] in which benzyl isothiocyanate, on basis of paper chromatography of the corresponding thiourea, is stated to be the sole mustard oil derivable from root material and fresh parts of the plant.

* Part L of a series of papers on naturally derived isothiocyanates. For part XLIX: see *Acta Chem. Scand.* **17** (1963) 846.

In our hands, paper chromatography of a methanolic seed extract of *Lepidium graminifolium* L.* revealed the presence of two thioglucosides, possessing R_B-values[3] of about 0.7 and 1.0 with butanol: acetic acid: water (12:3:5:) as a solvent system.** The former reacted with diazotized sulphanilic acid to give an intensely orange colour; the classical thioglucoside sinalbin, the only previously known phenolic member of this class of natural products, had a slightly lower R_B-value in the same solvent system and produced a brick-red colour with the diazo-reagent. The preliminary investigation therefore suggested the presence of at least one new thioglucoside in seed of *L. graminifolium*, the identification of which appeared to be of sufficient interest to warrant a more detailed investigation.

For this purpose, a larger quantity of seed was produced in the Botanic Garden of the University of Copenhagen.*** A crude thioglucoside-fraction was isolated from the defatted seed by ion exchange in the customary way (*cf. e.g.* Ref.[4]) and, without further purification, subjected to acid hydrolysis under conditions similar to those utilized by Ettlinger and Lundeen [5] for the hydrolytic fission of, *e.g.*, sinalbin to *p*-hydroxyphenylacetic acid. According to paper chromatography, the acid fraction of the hydrolysis mixture contained a component possessing the same R_F-value and producing the same light-brown colour with diazotized sulphanilic acid as a reference specimen of authentic *m*-hydroxyphenylacetic acid. Furthermore, the isomeric *o*- and *p*-hydroxyphenylacetic acids behaved distinctly different, the former by possessing a much higher R_F-value, and the latter by giving only a faintly yellow, initial reaction with the diazo reagent, slowly changing into a greyish-violet colour. The second acid, derived from the thioglucoside with the highest R_B-value, did not react with the diazotized amine.

In order to separate the two acids, they were converted into the corresponding methyl esters by simple Fischer esterification. The two esters were then separated by vapour phase chromatography with silicone rubber as the stationary phase. Mass spectrometry of the two methyl esters † provided useful information regarding their chemical structures. As apparent from Fig. 1, the two chromatographic fractions consisted of methyl esters of a ring-monohydroxylated phenylacetic acid and a corresponding *O*-methyl derivative, respectively. Although the mass spectra did not reveal the position of the substituents, it appeared likely, in view of the above paperchromatographic results and the previously reported occurrence of *m*-methoxybenzyl isothiocyanate in enzymically hydrolyzed plant material,[6] that we were here dealing with the methyl esters of *m*-hydroxyphenylacetic acid and its *O*-methyl

* The employed seed sample was kindly provided by the Botanic Garden of the University of Copenhagen.

** Traces of a third glucoside with an R_B-value of *ca.* 0.5 were noticed on most chromatograms.

*** The employed seed material was produced during the summers of 1961 and 1962 by cultivation of the plant on a larger scale in the experimental fields of the Botanic Garden of the University of Copenhagen. The authors are highly indebted to the Garden for this valuable assistance. The species has been botanically controlled on comparison with authentic herbarium specimens by Mr. K. Rahn whose help is also gratefully acknowledged.

† The authors are much indebted to Dr. C. Djerassi, Stanford University, U.S.A., and Dr. R. Ryhage, Karolinska Institutet, Stockholm, for the mass spectrometric determinations.

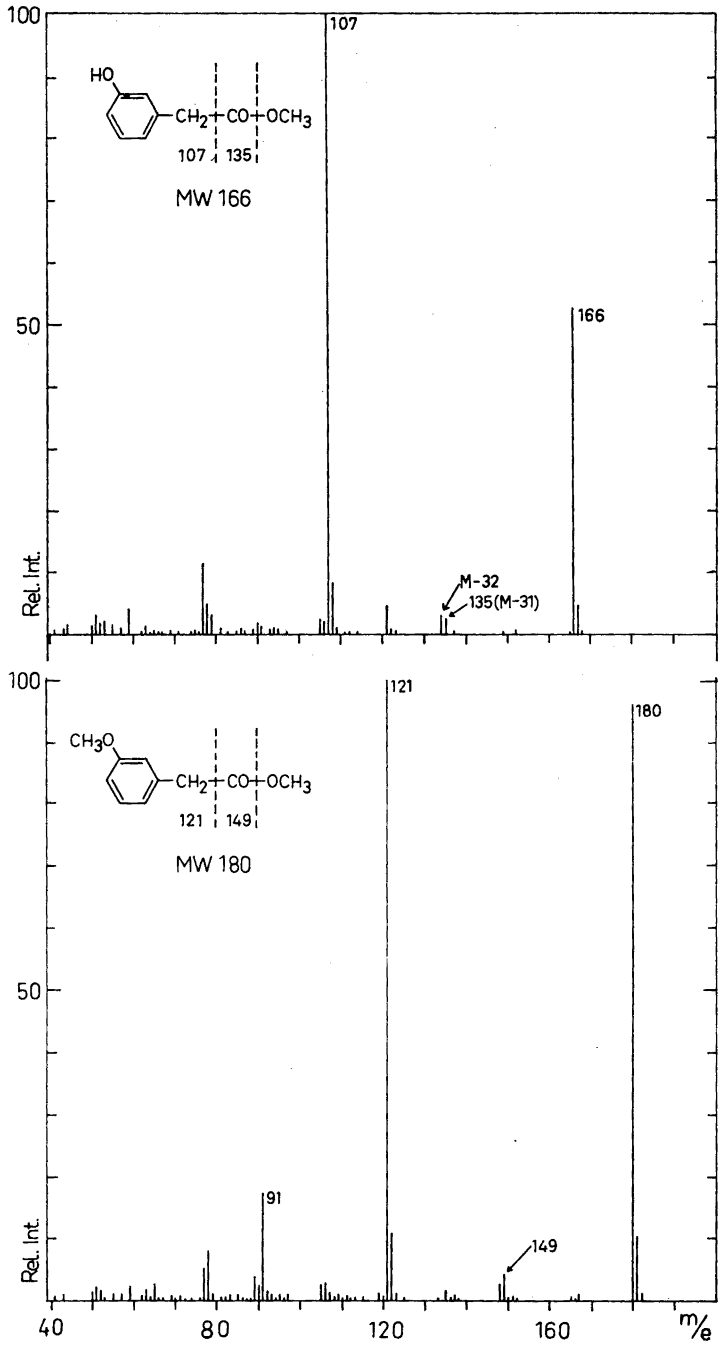
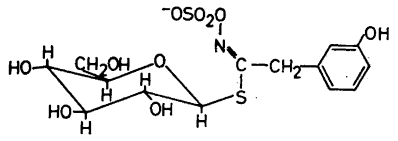


Fig. 1. Mass spectra of: methyl *m*-hydroxyphenylacetate (upper diagram), and methyl *m*-methoxy-phenylacetate (lower diagram).

derivative. This supposition was finally confirmed when both the infrared spectra and the mass spectra were proved to be identical with those of authentic, synthetic specimens of the methyl esters, produced by esterification of the known *m*-hydroxy- and *m*-methoxy-phenylacetic acids.

Consequently, seed of *L. graminifolium* L. contains two thioglucosides differing from each other only by a methyl group. Hence, the structural relationship is the same as that formerly established between sinalbin and glucoaubrietin.[7] The thioglucoside with the highest R_B-value, producing *m*-methoxyphenylacetic acid on acid hydrolysis, therefore seems identical with glucolimnanthin,* the glucosidic progenitor of *m*-methoxybenzyl isothiocyanate in seed of *Limnanthes Douglasii* R. Br. (meadowfoam), belonging to the small North American family Limnanthaceae.[6] It is of interest that the present identification of glucolimnanthin in a species of the family Cruciferae represents the first recognition of this thioglucoside outside the family Limnanthaceae. There seems to be no other alliance between these systematically remote families.

The second thioglucoside in *L. graminifolium* L. does not seem to have been recognized before. A solution of the new glucoside, produced by paper-chromatographic purification, was enzymically hydrolyzed by myrosinase to give, *inter alia*, sulphate ions and glucose, the latter identified by thin-layer chromatography.[8] Again, when another portion of the same solution was hydrolyzed with hydrochloric acid, hydroxylamine was produced. These reaction products are those expected for isothiocyanate-producing glucosides as demonstrated by Ettlinger and Lundeen.[5] Accordingly, the new glucoside



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is of the ordinary type, possessing the structure (I). No attempts were made in the present work to isolate the glucoside in pure form. According to the general practice for naming natural compounds of the present type (*cf.* Ref.[4]), the name *glucolepigramin* is proposed for the new glucoside ion (I).**

So far, glucolepigramin has been observed only in the species *L. graminifolia* L. When an extract of whole plants, kept in the dry state for several months, was analyzed by paper chromatography, neither the two seed glucosides, nor glucotropaeolin, the glucoside reported by Delaveau,[2] were observed, Instead, two glucoside spots of unidentified nature were observed in a much lower R_B-region.

* This name is conformal to other names within this class of natural products, but was not introduced by Ettlinger and Lundeen in their original report;[6] on proposal of one of the present authors (A.K.), however, Dr. M. G. Ettlinger has later agreed on this designation.

** In the semi-systematic nomenclature proposed by Ettlinger and Dateo,[9] the glucolepigramin ion is to be named: *m*-hydroxybenzylglucosinolate.

EXPERIMENTAL

Paperchromatographic analysis. A concentrated 70 % methanol extract of finely ground seeds of *Lepidium graminifolium* L. was applied, without further purification, to chromatography on Schleicher and Schüll 2043b paper with butanol:ethanol:water (12:3:5) as the solvent system. Comparison of chromatograms, run in parallel, but sprayed with ammoniacal silver nitrate and diazotized sulphanilic acid, respectively, indicated the presence in the seed of about equal amounts of two thioglucosides with R_B-values [3] of 0.7 and 1.0. The former gave an intense orange colour with the diazo-spray. Control chromatograms with authentic sinalbin showed that this thioglucoside migrated at a rate slightly lower than that of the unknown glucoside and produced a brick-red colour with the diazo-reagent.

Isolation and hydrolysis of the thioglucoside mixture. A large seed portion (250 g) was suspended in carbon tetrachloride and disintegrated and defatted in a Waring Blendor. Residual fatty material was removed by refluxing the powder with two subsequent portions of the same solvent. The fat-free residue (166 g) was extracted on reflux with two 1 l-portions of 70 % methanol, and the combined filtrates were evaporated to dryness. The dry residue (37 g) was dissolved in water (500 ml), and the thioglucoside-fraction was bound on a weakly basic ion exchange resin in the chloride form (Amberlite IR-4B, 30 × 3.5 cm). The column was thoroughly washed with water (7 l), and the thioglucosides were then eluted with 1 N sodium hydroxide. The glucoside-containing fractions were pooled, concentrated to dryness *in vacuo*, and the glucosides separated from sodium chloride by repeated extractions with hot, anhydrous methanol. The residual glucosidic fraction was dissolved in water, cautiously treated with charcoal, and the filtrate evaporated and dried. The glassy glucoside mixture (2.5 g) was used for degradation studies without further purification.

A portion of the glucoside mixture (1 g) was washed with two 10 ml-portions of ether, and then kept for 5 h in 20 % hydrochloric acid (25 ml) at 60°. The hydrolysis mixture was extracted with ether, and the acid hydrolysis products removed by extracting the ether solution with 5 % sodium hydrogen carbonate. After acidification, the acids were reextracted into ether. After drying, and removal of the solvent, a solid acid fraction (167 mg) was obtained.

On paper chromatography, with the bottom phase of the solvent system chloroform: acetic: acid: water (2:1:1) as the mobile phase, one spot appeared on spraying with diazotized sulphanilic acid. The initial, yellow colour changed within 17 h to a permanent light-brown spot. A reference sample of *m*-hydroxyphenylacetic acid, produced by demethylation with hydrogen iodide of *m*-methoxyphenylacetic acid according to literature directions,[10] behaved in exactly the same manner. The isomeric *o*-hydroxyphenylacetic and *p*-hydroxyphenylacetic acids * were distinctly different, the former by its much higher R_F-value and the latter by its colour reaction, changing from light yellow to greyish-violet within 17 h.

The production and vapour phase chromatography of the methyl esters. The above acid mixture (167 mg) was dissolved in methanol (20 ml), containing conc. sulphuric acid (150 µl), and the solution was refluxed for 3 h. The methyl ester fraction (94 mg) was isolated in the usual way.

Vapour phase chromatography was performed on an F & M 500-instrument, equipped with a hot wire detector and a silicone rubber column. The block was set at 235°, the injection port at 290°, and a column temperature programme of 5.6°/min was employed from a starting temperature of 125°. The helium flow was 60 ml/min. The two ester bands were separately collected in capillary tubes from repeated injections of the ester mixture. The mass spectra of the two esters (Fig. 1) gave strong molecular peaks at 166 and 180, respectively, with the corresponding benzyl ions at 107 and 121 as base peaks.

The mass spectra as well as the infra-red spectra of the two esters proved to be identical with those of methyl *m*-hydroxyphenylacetate and *m*-methoxyphenylacetate, synthesized by esterification of the corresponding acids as described below.

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* Both acids were commercial samples, purchased from California Corp. for Biochem. Research, Los Angeles 63, Calif., U.S.A.

Synthesis of methyl m-*hydroxyphenylacetate and* m-*methoxyphenylacetate.* *m*-Methoxyphenylacetic acid, synthesized from *m*-methoxyacetophenone via the Willgerodt-reaction,[11] was converted into the methyl ester upon reflux in methanol, with conc. sulphuric acid as a catalyst. The ester distilled as a colourless liquid, b.p. 145—146°/14 mm, n_D^{25} 1.5139 (Found: C 66.59; H 6.71. Calc. for $C_{10}H_{12}O_3$: C 66.64; H 6.71).

m-Hydroxyphenylacetic acid, prepared by demethylation of the methoxy-acid,[10] was esterified in the same way to give methyl *m*-hydroxyphenylacetate as a colourless oil, b.p. 107°/0.05 mm; n_D^{25} 1.5332, (Found: C 64.99; H 6.07. Calc. for $C_9H_{10}O_3$: C 65.06; H 6.07).

Degradation reactions of glucolepigramin. A solution of the glucoside mixture (145 mg) in water (1 ml) was distributed evenly over the starting lines of ten sheets of Whatman paper no. 3MM, and chromatographed for 18 h in butanol:ethanol:water (4:1:4). Edge cuts were utilized for locating the diazo-positive glucoside bands which were cut out and eluted with water. The resulting glucoside (20 mg) was divided into halves. Acid hydrolysis of one half, conducted in the usual way,[5] resulted in the liberation of hydroxylamine, identified by paper chromatography and spraying with picryl chloride.[5] The second half was subjected to enzymic hydrolysis with a sulphate-free myrosinase solution. After 3 h at room temperature, a positive sulphate reaction was obtained on a few drops of the reaction mixture. The remaining part was taken to dryness *in vacuo* and redissolved in pyridine. On thin layer chromatography, according to the procedure of Stahl and Kaltenbach,[8] glucose was easily identified as the sole sugar.

Microanalyses were performed by Mr. G. Cornali and his staff. The authors are much indebted to Dr. R. Gmelin, Dr. R. Boe Jensen, and Mrs. H. Thomsen for valuable assistance during the initial phases of the present work.

The work is part of investigations supported by *Carlsbergfondet (The Carlsberg Foundation)* and *Kai Hansen's Fond.*

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Received March 29, 1963.