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SHORT COMMUNICATION

A BITTER PRINCIPLE OF SAFFLOWER; MATAIRESINOL MONOGLUCOSIDE

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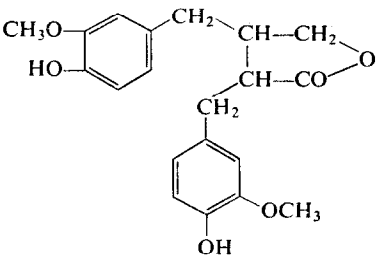
Abstract—During an examination of the components contributing to the bitter taste of safflower meal, *l*-matairesinol-mono-β-D-glucoside was isolated and identified. This represents the first observation of this lignan occurring naturally in glycosidic form.

INTRODUCTION

SAFFLOWER meal is being used exclusively as an animal feed. Its bitter taste and reported cathartic activity precludes it from being utilized for human food. Isolation and identification of the deleterious components of the meal was undertaken to determine methods for their removal as well as to study their pharmacological activity.

The first component to be isolated and identified is *l*-matairesinol-mono-β-D-glucoside. The aglycone *l*-matairesinol (I) had been isolated from the Matai tree (*Podocarpus spicatus*)



Matairesinol
(I)

of New Zealand as early as 1910[1] and its structure,[2,3] synthesis,[4] and configuration[5] have been known for many years. However, there is no previous report of its occurrence as a glycoside. As is characteristic of certain classes of compounds, glycosides have a bitter taste whereas their aglycones do not.[6] This is true for matairesinol and its glycoside. The procedures used for isolation and identification of this bitter component of safflower meal are reported.

[1] T. H. EASTERFIELD and J. BEE, *J. Chem. Soc.* **97**, 1028 (1910).
[2] L. H. BRIGGS, D. A. PEAK and J. L. D. WOOLLOXALL, *J. Roy. Soc. N. S. Wales* **69**, 61 (1935).
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[5] R. D. HAWORTH and L. WILSON, *J. Chem. Soc.* 71 (1950).
[6] R. M. HOROWITZ, in *Biochemistry of Phenolic Compounds*, pp. 545–571, Academic Press, New York (1964).

RESULTS AND DISCUSSION

Initially, the isolation of matairesinol monoglucoside required preparation of concentrates of bitter extracts of safflower meal. Fractionation of these concentrates revealed the presence of other bitter components, but work was primarily directed toward obtaining a sufficiently pure fraction of matairesinol monoglucoside. No attempt was made to quantitate the amount present in safflower meal.

To correlate bitterness with characteristic TLC bands from crude mixtures, the bands were scraped from the plate and water extracts tasted. From characteristic R_f, u.v. and anisaldehyde spray reagent band colors, it was possible to follow the course of purification. It was established that the bitter bands were glycosidic by testing with phenol-sulfuric acid;[7] this test was used for following column elution patterns.

Final isolation yielded the glycoside as a hygroscopic white powder, m.p. 92–102° $[\alpha]_D^{25}$ −57·9° in methanol. Identification of the compound as *l*-matairesinol-mono-β-D-glucoside proceeded from the following evidence. I.r. analysis indicated the presence of a lactone group by a sharp band at 1760 cm^{-1}. The u.v. spectrum, λ_{max} (ethanol) 282 mμ shifted to λ_{max} 302 mμ upon the addition of NaOH, suggesting the structure to be phenolic. The aglycone moiety resulting from hydrolysis showed a parent peak at *m/e* 358 in the m.s. and a molecular formula of $C_{20}H_{22}O_6$ was established by high resolution mass spectrometry.

Deuterated benzene was found to be the most useful proton NMR solvent for the aglycone since it minimized the overlapping of peaks. Integration of the NMR spectrum showed a total of 22 protons in agreement with the mass spectral analysis. The two peaks at 3·10 and 3·12 ppm indicated two aromatic methoxyl groups, a peak at 5·39 ppm showed hydroxyl (absent in the presence of D_2O) and a region from 6·0 to 7·0 ppm indicated two nonfused benzene rings, each with a 1,2,4-substitution pattern.

It appeared probable that the compound was a lignan, and the only known lignan possessing these characteristics was matairesinol. An authentic sample of *l*-matairesinol isolated from *Podocarpus spicatus* was obtained. TLC, NMR and i.r. comparison with the aglycone of the compound isolated from safflower showed the two to be identical. NMR spectra from the unknown and authentic *l*-matairesinol were almost identical. Very slight differences in the incompletely resolved NMR bands centered at 3·3 and 3·6 ppm are evident and may be due to a slight contamination by a diastereoisomer. The mass spectrum of the compound had been published previously[8] and matches the mass spectrum of the aglycone from the bitter principle of safflower meal.

The u.v. adsorption bands of the isolated aglycone and of the authentic matairesinol sample agree with the published matairesinol value.[9] Optical rotations (acetone) of the authentic sample of *l*-matairesinol of the aglycone obtained by acid hydrolysis and of the aglycone obtained by enzyme hydrolysis were $[\alpha]_D^{25}$ −37·9°, −35·7° and −40·5°, respectively.

The glycoside sugar from acid hydrolysis was found to be glucose by chromatographic comparison with authentic samples of several common sugars in two solvent systems. The β linkage was established by an enzymatic hydrolysis of the glycoside with β-glucosidase and by its 100 MHz NMR spectrum in dimethylformamide-d_7. A 6 Hz doublet (with additional incompletely resolved splittings) at 4·9 ppm with an area corresponding to one hydrogen is very characteristic of the anomeric proton in a β-glucoside. Assuming the compound to be a monoglucoside, both acidic and enzymatic hydrolysis gave an 85–90% yield of aglycone.

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[8] A. M. DUFFIELD, *J. Heterocyclic Chem.* **4**, 16 (1967).
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The glucose yield from acid hydrolysis was 90%. Further evidence supporting the assignment of a single glucose unit and a free phenolic group in the molecule comes from the u.v. adsorption shift of the glycoside with the addition of alkali. Thus, the identity of the compound has been established as *l*-matairesinol-mono-β-D-glucoside. It has not yet been determined which ring contains the glycosidic bond.

EXPERIMENTAL*

Extraction and Isolation Procedures

Oil-free safflower meal was continuously extracted with hot MeOH by percolation for 14 hr. The dried extract was then extracted with hot ethyl acetate for 6 hr and this extract was concentrated to dryness. After slurrying with H_2O and dialyzing for 24 hr, the extract material passing through the dialysis membrane was concentrated to a small volume, filtered and applied to a 50 mm × 95 cm ion exchange resin column (Bio Rad AG 50W-X2, 200–400 mesh, hydrogen form). The column was eluted with water at a flow rate 1·5 ml/min, and the effluent was monitored by u.v. and by test sample reaction with phenol-sulfuric acid. The portion of the effluent containing the desired compound (21·8–29·7 l.) was concentrated to dryness, dissolved in MeOH and applied to preparative TLC SiO_2 (Brinkmann, 2 mm) for development with $CHCl_3$–MeOH–H_2O (65:25:4). The relevant band area was determined under u.v. light, and that portion of SiO_2 was scraped from the plates, extracted with H_2O, dried and extracted with MeOH. Final purification to a white powder was achieved by application of the sample to a Sephadex LH-20 column, 50 mm × 144 cm, and eluting with MeOH at 2·35 ml/min; the effluent vol. was 2350–2438 ml.

Acid Hydrolysis

A 20 mg sample of the glycoside was hydrolyzed with 2 ml 0·5 N HCl for 30 min at 100° in an evacuated sealed tube. The aglycone was extracted from the acid mixture with $CHCl_3$ and applied to a preparative SiO_2 plate for development with benzene–EtOH (92:8). The aglycone band was located under u.v. light, extracted from the SiO_2 with $CHCl_3$ and dried.

The acid portion of the hydrolyzate mixture containing the sugar was passed through a small column of Duolite A-4 resin, and the neutral H_2O effluent dried. The sugar was identified as glucose on SiO_2 plates developed with PrOH–ethyl acetate–H_2O (70:20:10) and visualized with anisaldehyde spray. The sugar was also chromatographed on Whatman No. 1 paper, developed with BuOH–pyridine–H_2O (6:4:3); visualized with alkaline silver nitrate.

Enzyme Hydrolysis

β-Glucosidase solution (4 mg in 2 ml 0·1 M sodium acetate buffer, pH 5·0) was shaken with 20 mg of glycoside for 24 hr at 37°. The aglycone was extracted with $CHCl_3$ and purified by the same separation techniques as for the acid-hydrolyzed aglycone.

Instrumentation

Mass analysis was performed on a Consolidated Electrodynamics 21-110 Mass Spectrometer. A modified Varian HR 100 was used for the NMR spectrum. The i.r. spectrum was determined on a Perkin–Elmer 257 and the u.v. spectrum on a Cary 15. Optical rotations were taken on a Bendix 1100.

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* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.