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LINUSITAMARIN, A NEW PHENYLPROPANOID GLUCOSIDE FROM *LINUM USITATISSIMUM*

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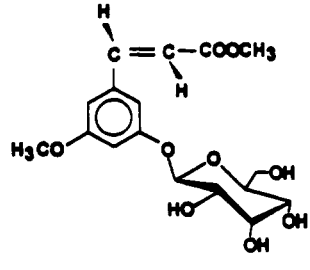
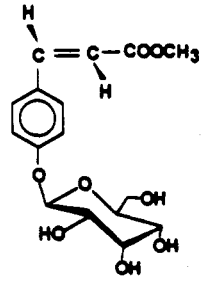
ABSTRACT.—From the defatted meal of flaxseed (*Linum usitatissimum*), a novel phenylpropanoid glucoside, linusitamarin [**1**], was isolated, along with a number of known compounds. The structure of **1** was determined by spectroscopic analysis.

Epidemiological studies have shown an inverse relationship between the dietary consumption of high-fiber foods such as flaxseed, *Linum usitatissimum* L. (Linaceae), and the incidence of breast and colon cancers (1). Included among the constituents of flaxseed are the unsaturated fatty acids linoleic and linolenic acids (2,3), and the simple linear lignan secoisolariciresinol diglucoside (4). Recent reports on the roles of this lignan in chemoprevention have been reviewed (1,5,6). In essence, secoisolariciresinol diglucoside or the aglycone is converted by the intestinal flora of animals to the metabolites enterodiol [2,3-bis(3-hydroxybenzyl)butane-1,4-diol] and enterolactone [*trans*-2,3-bis(3-hydroxybenzyl)-γ-butyrolactone], which are structurely similar to diethylstilbesterol, but without estrogenic activity (7). These metabolites have been postulated to possess antiestrogenic properties (1).

In response to a U.S. National Cancer Institute initiative, we undertook a study on the modulatory influence of dietary flaxseed on the metabolic fate of secoisolariciresinol diglucoside in humans. The concentration of lignan in flaxseed-containing diets or biological fluids of the study subjects was analyzed. The glycoside was obtained as a reference compound by extracting flaxseed with 1,4-dioxane–EtOH (1:1) following the method of Bakke and Klosterman (4). A light powder was obtained. Treatment of this material with an anhydrous MeOH solution of NaOMe, followed by chromatography, afforded secoisolariciresinol diglucoside, together with linocinnamarin [**2**], daucosterol, and a new phenylpropanoid glucoside to which we gave the name linusitamarin [**1**].



1



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Linusitamarin [**1**] was obtained as an amorphous powder from MeOH. It displayed a quasi-molecular ion of *m/z* 371.1349 (hrfabms), corresponding to a molecular formula of $C_{17}H_{22}O_9$. The uv spectrum (λ max 232, 290.5, 318 nm, without bathochromic shift in alkali) of **1** resembled that of the phenylpropanoid linocaffein (8), and the ir data revealed an absorption band at 3451 cm^{-1} due to OH groups.

The ^{13}C-nmr spectrum of **1** (Table 1) showed 17 carbon signals. A standard APT pulse sequence was employed to delineate the carbon resonances as two Me, one methylene, ten methine, and four quaternary carbons. The presence of a glucose moiety could be readily identified from the spectrum (9). Furthermore, a comparison of the ^{13}C-nmr data of **1** with those of linocinnamarin [**2**] indicated that **1** is a cinnamic acid Me ester. Thus, two olefinic carbon signals at δ 116.5 and 146.1 were assigned to C-α and C-β, respectively. The corresponding proton signals and coupling constant (J=16 Hz) suggested a *trans* configuration of the double bond. The remaining carbon signal (δ 56.8) represented a second MeO group. It followed that the aromatic ring was trisubstituted, and a 1,3,5-trisubstitution pattern was suggested because all aromatic protons appeared as broad singlets in the ^{1}H-nmr spectrum (Table 1).

Assignment of the nmr spectral data was accomplished by HETCOR and selective INEPT experiments. Selective INEPT irradiation of the anomeric proton (δ 4.98) enhanced the carbon signal at δ 150.1, thereby establishing C-3. It followed that the quaternary carbon signal at δ 151.0 was due to C-5. Evidence for the assignment of C-2, C-4, and C-6 was obtained from further selective INEPT studies. Polarization transfer from H-β enhanced the carbonyl (δ 169.2), C-2/C-6 (δ 112.6 and 123.5), and C-α (δ 116.5, 2-bond coupling). This also allowed the assignment of the signal at δ 117.4 to C-4. Selective INEPT irradiation of the proton singlet at δ 7.24 (shown by HETCOR to be connected to the carbon peak at δ 112.6), resulted in the enhancement of carbons at δ 150.1 (C-3, 2-bond coupling) and 123.5, permitting the assignment of the latter signal to C-6. The carbon signal at δ 112.6 was thus C-2. Compound **1** was established as methyl 3-β-D-glucopyranosyl-5-methoxycinnamate.

A related compound, linocinnamarin

TABLE 1. ^{13}C- and ^{1}H-nmr Spectral Data of Compounds **1** and **2**.

Position	Compound			
	1 δ_C (ppm)	**2** δ_C (ppm)	**1** δ_H (ppm)	**2** δ_H (ppm)
1	130.4	127.7	—	—
2	112.6	129.9	7.24 (s)	7.07 (d, J=9 Hz)
3	150.1	116.1	—	7.69 (d, J=9 Hz)
4	117.4	159.1	7.16 (s)	—
5	151.0	116.1	—	7.69 (d, J=9 Hz)
6	123.5	129.2	7.16 (s)	7.07 (d, J=9 Hz)
α	116.5	115.5	6.43 (d, J=16 Hz)	6.54 (d, J=16 Hz)
β	146.1	144.1	7.63 (d, J=16 Hz)	7.66 (d, J=16 Hz)
COOMe	169.2	166.8	—	—
COO*Me*	52.1	51.2	3.67 (s)	3.37 (s)
ArO*Me*	56.8	—	3.88 (s)	—
1′	102.2	99.9	4.98 (d, J=7 Hz)	4.96 (d, J=7 Hz)
2′	74.8	73.1	3.2–3.7	3.1–3.9
3′	77.8	76.5	3.2–3.7	3.1–3.9
4′	71.2	69.6	3.2–3.7	3.1–3.9
5′	78.2	77.0	3.2–3.7	3.1–3.9
6′	62.5	60.6	3.7–3.9	3.1–3.9

[**2**], was also obtained from the flaxseed extract. This compound was first described by Klosterman *et al.* (10). Complete assignments of the ^{1}H- and ^{13}C-nmr data for **1** and **2** are given in Table 1.

MeOH extraction of flaxseed also yielded a number of known compounds, including β-sitosterol, oleic acid, nicotinamide, neolinustatin, linustatin, and linoleic Me ester.

EXPERIMENTAL

GENERAL PROCEDURES.—Uv measurements were performed in MeOH using a Beckman DU-7 spectrometer, and ir spectra were recorded with a Nicolet MX-1 interferometer. ^{1}H-nmr data were obtained in CD_3OD with TMS as internal standard on a Varian XL-300 spectrometer operating at 300 MHz. ^{13}C-nmr, HETCOR, and selective INEPT experiments were recorded on the same instrument at 75.44 MHz. A Varian MAT 112S mass spectrometer was used for low resolution eims, and hrfabms was recorded on a Finnigan MAT 90 spectrometer.

PLANT MATERIALS.—Samples of pulverized flaxseed were obtained from Arthur D. Little Co. (Acorn Park, Cambridge, MA), with the following specifications: product code FFFLX 900000 (Essential Nutrient Research Co., Manitowoc, WI); ingredients flax, $ZnSO_4$, vitamin B-6; protein 24%, fat 40%, carbohydrate 10%, fiber 25%.

EXTRACTION AND ISOLATION.—The initial sample of flaxseed (5.16 kg) was extracted with MeOH (30 liters). The extract was concentrated in vacuo to a syrup and partitioned between petroleum ether-MeOH-H_2O (5:1:4) to yield an oily petroleum ether fraction. The aqueous MeOH solution was then partitioned with $CHCl_3$ to give fraction 2 (10 g). The aqueous layer was further partitioned with *n*-BuOH to give an *n*-BuOH fraction (fraction 3, 18 g). Cc of fraction 2 on Si gel, eluting with $CHCl_3$ and MeOH mixtures of increasing polarity (0–20% MeOH), resulted in the isolation of β-sitosterol (100 mg), oleic acid (84 mg), and nicotinamide (195 mg). A similar cc of fraction 3 afforded crops of neolinustatin (100 mg), linustatin (25 mg), sucrose (2 g), a mixture of *cis*- and *trans*-linoleic acid methylester (45 mg), and tryptophan (204 mg). The identities of these isolates, except neolinustatin and linustatin (11), were confirmed by direct comparison (co-tlc, ^{1}H and ^{13}C nmr, ms) with authentic samples available in our laboratories.

Since the previous separation failed to yield secoisolariciresinol diglucoside, a second sample of flaxseed meal (5.02 kg) was extracted using a modification of the method of Bakke and Klosterman (4). The material was defatted with petroleum ether, followed by further defatting with $CHCl_3$. The marc was then exhaustively extracted with dioxane-EtOH (1:1). A sample (60 g) of the plastic-like powder remaining following in vacuo evaporation of the solvent was treated with NaOMe (4 g) in anhydrous MeOH (250 ml) by constant stirring for 48 h. The resulting mixture was concentrated, and the pH was adjusted to 3.0 with 1 N H_2SO_4; the mixture was then chromatographed on a Si gel column packed in $CHCl_3$. Development was accomplished by eluting with the lower phase of a solvent mixture containing $CHCl_3$-MeOH-H_2O (65:35:10). Workup of the resulting fractions led to the isolation of secoisolariciresinol diglucoside (10.2 g), daucosterol (15 mg), linocinnamarin [**2**] (150 mg), and the new compound linusitamarin [**1**] (25 mg).

Linusitamarin [**1**].—Amorphous powder: uv (MeOH) λ max 232, 290.5, 318 nm; ir (KBr) ν max 3451, 2932, 1713, 1638, 1599, 1512, 1264 cm^{-1}; ^{1}H and ^{13}C nmr, see Table 1; hrfabms *m/z* $[M+1]^+$ 371.1349 ($C_{17}H_{22}O_9$, calcd 371.1342); eims *m/z* $[M-C_6H_{10}O_5]^+$ 208, $[208-OMe]^+$ 177.

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