Qatar Univ. Sci. J. (1992), 12: 69 - 72

CONSTITUENTS OF GREEN BEANS *PHASEOLUS VULGARIS* (LIPIDS AND FLAVONOIDS)

By

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مكونات الفاصوليا الخضراء
(الدهنيات والفلافونيدات)

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و صفاء أحمد عزام و ج . وود

أسفرت دراسة الدهنيات عن فصل والتعرف على هيدروكربونات مشبعة ، كحولات أليفاتية مشبعة وستيرويدات (بيتا سيتو ستيرول ستيجما ستيرول) وأسفر تحليل الأحماض الدهنية عن وجود احماض الميرستيك ، الهكساديكانويك ، الستياريك ، الأوليك ، اللينوليك .

وقد اسفرت دراسة المواد الفلافونيدية عن التعرف على مركبين . كامبفيرول - ٣ - روتينوسيد ، كيرستين - ٣ - روتينوسيد .

Key Words: Phaseolus vulgaris, Leguminosae, Flavonoids, Kaempferol-3-rutinoside, Quercetin-3-rutinoside, Hydrocarbons, Aliphatic alcohols, Sterols, Fatty acids.

ABSTRACT

Chemical study of the lipid fraction resulted in the isolation and identification of a hydrocarbon fraction (n-C_{29} -n-C_{33}); an aliphatic alcohol fraction (C_{28}, C_{29}, C_{30}) and a sterol fraction (stigmasterol and sitosterol). Analysis of the fatty acids revealed the presence of myristic, palmitic, hexadodecanoic, stearic, oleic and linoleic acids. The flavonoid constituents were identified as kaempferol-3-rutinoside and quercetin-3-rutinoside.

INTRODUCTION

The green beans *Phaseolus vulgaris*, family Leguminosae is widely consumed by human. In recent years several studies have been conducted on green beans. A large number of these studies have been concerned with the ability of beans to produce various defensive compounds when they are attacked in the field or in storage by certain micro-organisms (Rizk and Wood, 1980).

The phytoalexins produced after inoculation of green bean pods with spore suspensions of three fungi have been studied by the authors. Five phytoalexins were isolated and identified as phasseollin, coumestrol, phaseollidin, kievitone and 6 ∝ -hydroxyphaseollin (Rizk *et al,* 1984).

Other studies have included the chemical analysis of various tissues of healthy bean plant, but nothing has been reported on the chemical constituents of the pods.

The leaves and seeds were reported to contain flavonoids (March, 1955; Schwarze, 1959; Harborne, 1965; Rathmell and Bendall, 1971; Dikhtyarev *et al,* 1986), saponins (Chirva *et al,* 1970; Lazur'evskii *et al,* 1971), organic aicds (Bentley, 1952; Bakowski *et al,* 1964) and sterols (Duperon, 1964; Aoki *et al,* 1965).

EXPERIMENTAL

Plant material:

Fresh healthy green beans *Phaseolus vulgaris* L. were carefully selected and purchased at a local market. The seeds were removed from the pods. The pods were lyophilized and ground into powder form.

Thin-layer chromatography:

Lipids:

Silica gel G, developed with benzene - acetone (6:2); spraying reagent : 5% sulphuric acid, followed by heating at 120°C for 5-10 minutes.

Flavonoids:

a -Silica gel G F_{254}, developed with chloroform-methanol-formamide (80:19:1); *b*-Polyamide, developed with *n*-butanol-acetic acid-water (6:2:1).

Paper chromatography:

Flavonoids:

Whatmann 3 MM paper chromatogram sheets, developed with 15% acetic acid, applying the descending technique. Elution of the flavonoidal components was performed by 70% methanol.

Sugars:

Whatmann No. 1; developed with *n*-butanol-acetic acid-water (4:1:5); spraying reagent: aniline phthalate.

Gas-liquid chromatography:

Hydrocarbons:

Appraratus: Perkin-Elmer Sigma 3 instrument; Column: 1.8 m. x 2 mm. i.d. stainless steel, packing: 2% S.E-30 on gas chromosorb Q (80-100 mesh); carrier gas: helium (flow rate 60 ml./min.); temperature: programmed 125-250°C, 5°C/min; detector: FID.

Alcohols:

Column: 12.5 m. x 0.2 mm HP flexible capillary column, packing: liquid phase OV-1, immobilized by chemical cross-linking; carrier gas: hydrogen (flow rate 10 PSI); temperature: 250°C (3 min.) programmed up to 290°C (10°C/min.).

Sterols:

Apparatus: Perkin Elmer Sigma 3 instrument; column; 6 ft. x 1/8 inch. i.d. stainless steel column; packing 1% X E-60 om Anakrom ABS (90-100 mesh); carrier gas: helium (flow rate 50 ml./min.); temperature: 250°C, detector: FID.

Fatty acids:

Column: 6 ft x 1/8 inch. i.d. glass column; packing: 20% DCGS on chromosorb W-AW (80-100 mesh); carrier gas: nitrogen, (flow rate 25 ml./min.); temperature: 190°C; detector: FID.

High-performance liquid chromatography:

Sterols:

Apparatus: Dupont, column: Dupont zorbax C_{18}; solvent: acetonitrile-isopropanol (80-20) (flow rate 1 ml./min.); detector UV at 254 nm. sensitivity AUFS 2.0; temperature: 40°C.

Mass spectrometer:

Mass spectra were obtained on A.E.I. M S-902 spectrometer.

I. Lipid fraction

About 300 gm of the dried pods were extracted with petroleum ether in a soxhlet extractor for 20 hours. The greenish extract was decolourized by shaking with Fuller's earth, filtered and evaporated to dryness (2.7 gm.).

The residue (2.5 gm.) was chromatographed on silica gel (70-230 mesh). Elution was affected with petroleum ether (300 ml.), chloroform (500 ml.) and acetone (300 ml.), collecting 100 ml. fractions. The acetone fraction (1 gm.) was re-chromatographed on neutral aluminium oxide. Elution was carried out with petroleum ether, followed by increasing amounts of diethyl ether in petroleum ether.

Hydrocarbon fraction

Crystallization of the residue obtained from the petroleum ether fraction of both columns, afforded a substance which melted at 64°C. GLC analysis revealed that it is a mixture of *n*-alkanes Table 1.

Alcohol fraction

An alcohol mixture was obtained from fractions 8-11 of the petroleum ether-diethyl ether (90-10) eluate, from the neutral aluminium oxide column. Crystallization from chloroform/methanol gave white plates m.p. 73°C. GLC analysis was carried out and showed that it is a mixture of aliphatic alcohols.

Sterol fraction

The residue obtained from fractions 14 and 15 eluted with petroleum ether-diethyl ether (90:10) from the aluminium oxide column, gave upon crystallization form methanol/chloroform, white needles m.p. 135-136°C. It was further purified by precipitation with digitonin (Burchfield and Storrs, 1962) and subjected to both GLC (as trimethylsilyl ether and HPLC analyses). The trimethylsilyl ether was prepared as follows: About 25 mg of the purified sterol, dissolved in anhydrous tetrahydrofuran, was heated with 0.5 ml. hexamethyldisilazone and 10 ml. trimethyl-chlorosilane - in a stoppered tube at 55°C for 30 minutes.

Fatty acids

About 0.2 gm. of the total lipid fraction was saponified with 0.5 N KOH in ethanol. The fatty acids (0.053 gm.) obtained (after removal of the unsaponifiable matter) in the usual manner, were subjected to GLC after conversion to their methyl esters Table 2.

II. Flavonoids

About 950 gm. of the dried pods were defatted with petroleum ether, followed by extraction with methanol for 36 hours. The methanolic extract was evaporated to dryness, the residue was dissolved in hot distilled water (300 ml.), stored at 4°C overnight and refiltered. The filtrate was extracted with several portions of chloroform, followed by ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulphate then evaporated. The residue (0.72 gm.) was subjected to column chromatographic fractionation using polyamide. Elution was affected with chloroform, followed by mixtures containing increasing amounts of methanol in chloroform, collecting 10 ml. fractions. Some of the fractions were subjected to preparative PC.

Kaempferol-3-rutinoside

Fractions eluted with chloroform-methanol (60:40), after subjecting to preparative PC afforded yellow crystalline substance which melted at 222-224°C. It was proved to be kaempferol-3-rutinoside according to m.p., TLC, PC and UV spectral analysis (Geissman, 1962; Mabry *et al*, 1970). Moreover, acid hydrolysis (10% H_2SO_4), gave an aglycone, which upon crystallization from methanol, melted at 278-280°C and was identified as kaempferol (m.p., PC, UV and MS). The sugar moiety was

identified by PC., as glucose and rhamnose.

Rutin (quercetin-3-rutinoside)

Fractions eluted with chloroform-methanol (30:70) were further subjected to preparative PC. Crystallization from methanol/water gave pale yellow crystals, m.p. 187-190°C and was proved to be rutin (m.p., TLC, PC, and UV). Moreover, acid hydroysis (H_2SO_4), gave an aglycone which upon crystallization from methanol, melted at 314-317°C and was identified as quercetin (m.p., TLC, UV and MS). The sugar moiety was identified as glucose and rhamnose.

RESULTS AND DISCUSSION

Fractionation of the lipid fraction by column chromatography succeeded in the isolation of a hydrocarbon, an alcohol and a sterol fractions. The hydrocarbon fraction was shown by GLC to be a mixture of *n*-alkanes Table 1 (*viz.* *n*-$C_{29}H_{60}$(8%); *n*-$C_{30}H_{62}$ (1%); *n*-$C_{31}H_{64}$ (80%); *n*-$C_{32}H_{66}$ (1%) and *n*-$C_{33}H_{68}$ (10%). The aliphatic alcohol fraction was shown by GLC to be a mixture of long chain fatty alcohols (C_{28}-C_{30}); of these $C_{30}H_{62}O$ represents the major component (R_t 7.46 min.).

Table 1
The percentages of *n*-alkanes in the hydrocarbon fraction.

n-alkane	Percentage
$C_{29}H_{60}$	7.9
$C_{30}H_{62}$	1.13
$C_{31}H_{64}$	79.88
$C_{32}H_{66}$	1.12
$C_{33}H_{68}$	9.97

GLC analysis of the trimethylsilyl ether derivative of the sterol fraction showed that it is a mixture of stigmasterol (66%) and β-sitosterol (34%). Moreover, HPLC analysis confirmed these results, (stigmasterol, 69%; β-sitosterol, 31%).

GLC analysis of the fatty acids (26% of the total lipids) revealed the presence of myristic, palmitic, hexadecanoic, stearic, oleic and linoleic acids Table 2.

Table 2
The fatty acids of *phaseolus vulgaris*

Fatty Acid	Percentage
Myristic	0.27
Palmitic	28.30
Hexadecanoic	1.79
Stearic	4.65
Oleic	32.73
Linoleic	32.69

The method used for the preparation of the flavonoids involved the extraction of the defatted plant material with methanol and after concentration, extractions with chloroform and ethyl acetate. TLC on both silica gel and polyamide as well as PC revealed that the ethyl acetate fraction contained most of the flavonoidal constituents. The combination of both column chromatography and preparative PC succeeded in the isolation of the two prominent flavonoidal components in a pure form, which were identified as kaempferol-3-rutionside and quercetin-3-rutinoside.

Their identity was proved by comparison of their m.p., R_f, UV spectral data Table 3, IR and MS with that reported for authentic compounds. This was confirmed by acid hydrolysis and identification of their aglycones as kaempferol and quercetin and the sugar moieties as rhamnose and glucose.

Table 3
UV spectra of the isolated flavonoids

Additions to methanol	max. (nm)			
	Kaempferol		Quercetin 3-rutinoside	
	Band I	Band II	Band I	Band II
None	277, 316	252 sh, 267	301 sh, 358	258, 264 sh
NaOMe	414 (dec.)	277, 316	326 sh, 412	273
$AlCl_3$	301 sh, 351, 422	255 sh, 268	305 sh, 430	276
$AlCl_3$/HCl	300 sh, 351, 422	255 sh, 268	364 sh, 400	272, 299 sh
NaOAc	301, 386	275	324, 391	270
NaOAc/H_3BO_3	318 sh, 371	267, 297 sh	296, 385	261

ACKNOWLEDGEMENT

The authors are deeply indebted to the Food and Drug Administration (US-FDA) for the financial support of this work as a part of the project entitled "Poisonous Plants Contaminating Edible Ones and Toxic Substances in Plant Foods". The authors wish to express their gratitude and deep thanks to Prof. Dr. M.D. Sayed, Faculty of Pharmacy, Cairo University and Prof. Dr. F.M. Hammouda Pharmaceutical Science Department, National Research Centre, Cairo, for their indispensable help. Thanks are also expressed to Prof. Dr. M. Guthardt, Institute, fuer Pflanzenbiologie, Zuerich, for executing GLC analysis.

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