The UV spectrum (λ_{max} = 335 nm in $CHCl_3$) showed that the double bonds are not in conjugation with the carbonyl group.

Product III was identified as 9-oxononanoic acid from the following results: The methyl ester was coincident with the authentic methyl 9-oxononanoate on GLC and in the features of the mass spectrum. Product III formed a DNPH derivative (λ_{max} = 335 nm in $CHCl_3$). After methylation the DNPH derivative of III gave a mass spectrum with an intense molecular ion at m/e 366. The spectrum was identical with that of the corresponding reference substance.

DISCUSSION

A relatively stable hydroperoxide lyase showing optimal activity at pH 6.5 was partially purified from pears. The enzyme is the first example of a hydroperoxide lyase which cleaves exclusively the 9-hydroperoxides of linoleic and linolenic acid. Its substrate specificity contrasts with that of the specific hydroperoxide lyases mentioned in the introduction which are only active against the corresponding 13-hydroperoxides.

Incubation of the HL fraction with 9-HPOD yields *cis*-3-nonenal and 9-oxononanoic acid as major products and relatively small amounts of *trans*-2-nonenal. The trans isomer may be formed from *cis*-3-nonenal either by a nonenzymatic rearrangement or by the action of *cis*-3,-*trans*-2-enal isomerase which has been found, e.g., in cucumber fruit (Phillips et al., 1979) and tea leaves (Hatanaka and Harada, 1973). From the difference in the amounts of both nonenals formed during incubation of the HL fraction with 9-HPOD, we conclude that the isolated hydroperoxide lyase might be possibly contaminated with a small amount of isomerase activity.

LITERATURE CITED

Beisenherz, G. H.; Boltze, J.; Bücher, Th.; Czok, R.; Garbade, K. H.; Meyer-Arendt, E.; Pfleiderer, G. *Z. Naturforsch., B* **1953**, *8B*, 555.
Chan, H. W.-S.; Prescott, F. A. A. *Biochim. Biophys. Acta* **1975**, *380*, 141.
Egmond, M. R.; Brunori, M.; Fasella, P. M. *Eur. J. Biochem.* **1976**, *61*, 93.
Galliard, T.; Matthew, J. A. *Phytochemistry* **1977**, *16*, 339.
Galliard, T.; Phillips, D. R.; Reynolds, J. *Biochim. Biophys. Acta* **1976**, *441*, 181.
Gardner, H. W. *J. Agric. Food Chem.* **1975**, *23*, 129.
Grosch, W. *Z. Lebensm.-Unters. -Forsch.* **1976**, *160*, 371.
Grosch, W. *Z. Lebensm.-Unters. -Forsch.* **1977**, *163*, 4.
Grosch, W.; Laskawy, G.; Fischer, K.-H. *Lebensm.-Wiss. Technol.* **1974**, *7*, 335.
Hatanaka, A.; Harada, T. *Phytochemistry* **1973**, *12*, 2341.
Hatanaka, A.; Kajiwara, T.; Sekiya, J.; Fujimura, K. *Agric. Biol. Chem.* **1979**, *43*, 175.
Hatanaka, A.; Sekiya, J.; Kajiwara, T. *Phytochemistry* **1978**, *17*, 869.
Kim, I.-S.; Grosch, W. *Z. Lebensm.-Unters. -Forsch.* **1978**, *167*, 324.
Kim, I.-S.; Grosch, W. *J. Agric. Food Chem.* **1979**, *27*, 243.
Matthew, J. A.; Chan, H. W.-S.; Galliard, T. *Lipids* **1977**, *12*, 324.
Matthew, J. A.; Galliard, T. *Phytochemistry* **1978**, *17*, 1043.
Meijboom, P. W. *Fette, Seifen, Anstrichm.* **1968**, *70*, 477.
Phillips, D. R.; Galliard, T. *Phytochemistry* **1978**, *17*, 355.
Phillips, D. R.; Matthew, J. A.; Reynolds, J.; Fenwick, G. R. *Phytochemistry* **1979**, *18*, 401.
Schieberle, P.; Grosch, W. *J. Am. Oil Chem. Soc.* **1981**, *58*, 602.
Schlenk, H.; Gellerman, J. L. *Anal. Chem.* **1960**, *32*, 1412.
Schormüller, J.; Walther, M.; Wachs, W. *Z. Lebensm.-Unters. -Forsch.* **1969**, *139*, 273.
Sekiya, J.; Kajiwara, T.; Hatanaka, A. *Agric. Biol. Chem.* **1979**, *43*, 969.
Stein, R. A.; Nicolaides, N. *J. Lipid Res.* **1962**, *3*, 476.
Tressl, R.; Drawert, F. *J. Agric. Food Chem.* **1973**, *21*, 560.
Vick, B. A.; Zimmerman, D. C. *Plant Physiol.* **1976**, *57*, 780.
Wardale, D. A.; Galliard, T. *Phytochemistry* **1975**, *14*, 2323.

Received for review March 6, 1981. Accepted July 30, 1981.

Sterols, Esterified Sterols, and Glycosylated Sterols of Cow Pea Lipids (*Vigna uguiculata*)

Vhundi G. Mahadevappa and Piyara L. Raina*

Four sterol-containing lipid fractions, viz., free sterol, esterified sterol, sterol glycoside, and esterified sterol glycoside, were isolated from the chloroform–methanol extracted lipids of cow pea by preparative column and thin-layer chromatography. On a total lipid basis, these comprised 0.13%, 0.024%, 0.036%, and 0.029%. The major fatty acids in both the esterified fractions were linoleic, linolenic, and palmitic acids. Esterified sterol was more unsaturated (calculated iodine value of 139) than esterified sterol glycoside (calculated iodine value of 93). All the four sterol lipids contained high proportions of β-sitosterol and stigmasterol. About 3% campesterol has also been reported. The sugar identified in both the glycosylated sterols was only D-glucose. On the basis of the findings, the major representative species deduced are as follows: esterified sterols, β-sitosterol/stigmasterol linoleate, and to a lesser extent linolenate and palmitate; sterol glycoside, β-D-glucopyranosyl-(1→3)-S where S represents either β-sitosterol or stigmasterol; esterified sterol glycoside, 6-*O*-acyl-β-D-glucopyranosyl-(1→3)-S where S again represents either β-sitosterol or stigmasterol.

Cow pea (*Vigna uguiculata*) is one of the very important legumes grown and consumed as a source of protein in the southern part of India. Lipid composition of this legume has been reported earlier (Mahadevappa and Raina, 1978a). These workers have further shown that the lipids extracted from the legume had a hypocholesterolemic effect in rats and rabbits maintained on atherogenic diets which may stem from the considerable levels of polyunsaturated fatty acids (Mahadevappa, 1980). This legume also carries high proportions of sterol-containing lipids.

Discipline of Biochemistry and Applied Nutrition, Central Food Technological Research Institute, Mysore 570 013, India (P.L.R.), and Department of Biochemistry, Karnatak Medical College, Hubli 580 022, India (V.G.M.).

0021-8561/81/1429-1225$01.25/0

Table I. Fatty Acid Composition of Esterified Sterol and Esterified Sterol Glycoside (Percent Weight)

fatty acid	esterified sterol fraction	esterified sterol glycoside fraction
12:0	1.0	1.0
14:0	1.4	2.0
16:0	24.5	38.0
18:0	4.0	5.4
18:1	6.1	5.2
18:2	42.6	30.0
18:3	20.4	18.4
calcd iodine value of fatty acids	139	93

Keeping in view the importance of sterols and sterol-containing lipids in dietary sources, an attempt has been made to isolate sterol compounds present in cow pea seed lipids in pure form and identify the associated chemical constituents. The present paper describes the isolation and chemical constituents of four sterol compounds.

EXPERIMENTAL SECTION

Materials. A high-yielding variety of cow pea C-152 (*V. uguiculata*) was supplied by the University of Agricultural Sciences, Hebbal, Bangalore.

Lipid Extraction, Purification, and Fractionation. Extraction and purification of lipids from the ground cow pea were done essentially according to the procedures described in the earlier communication (Mahadevappa and Raina, 1978a). The purified lipid was resolved into neutral lipids, glycolipids, and phospholipids on a silicic acid column by sequential elution with chloroform, acetone, and methanol (Rouser et al., 1967). Total recoveries amounted to 85–90%.

Isolation of Sterol Lipids. The four sterol lipids from the neutral and glycolipids, two from each, were isolated by silicic acid column and preparative thin-layer chromatography as for the sterol lipids of finger millet seeds (Mahadevappa and Raina, 1978b).

Infrared Spectroscopy. Infrared spectral characteristics of the isolated compounds were obtained on a Hilger Watts infragraph (1 mg of sterol compound as 75 mg of potassium bromide).

Degradation of Sterol Lipids. The individual components of esterified sterols, sterol glycosides, and esterified sterol glycoside were obtained and subsequently purified for further identification by adopting the procedure described earlier (Mahadevappa and Raina, 1978b).

Paper Chromatography. Methyl glycosides prepared from sterol glycoside and esterified sterol glycoside were subjected to descending paper chromatography on Whatman No. 2 filter paper using 1-butanol–pyridine–water (6:4:3) for 18 h at room temperature. Spots were located with silver nitrate–sodium hydroxide reagent.

Gas Chromatography. Fatty acid methyl esters were analyzed by gas chromatography using a Varian Aerograph 1400 [flame ionization detector; nitrogen as the carrier gas; 8 ft × $^1/_8$ in. column with 15% (w/w) diethylene glycol succinate polyester coated on Chromosorb W; 185 °C]. For resolution of trimethylsilyl ether derivatives of sterols and methyl glycosides (Carter and Gaver, 1967), an SE-30 column (5% w/w) on Chromosorb W at 230 °C was employed. Unknown peaks were identified by comparison with those of standards. Unsaturated fatty acids were confirmed by bromination and subsequent disappearance on gas chromatograms.

RESULTS

The four sterol compounds isolated from neutral lipids and glycolipids, two from each, gave a single spot corresponding to reference sterols, esterified sterols, sterol glycoside, and esterified sterol glycoside, suggesting that the compounds isolated were essentially pure. Sterols, sterol glycoside, and esterified sterol glycoside gave an IR absorbance characteristic of the hydroxyl group at 3440 cm^{-1} but not the esterified sterol fraction. In esterified sterols and esterified sterol glycoside fractions, a strong absorbance at 1730 cm^{-1} which is characteristic of C=O was observed. Broad absorbance in the region of 1125–1000 cm^{-1}, characteristic of sugar, occurred in the spectra of sterol glycoside and esterified sterol glycoside. These characteristics are in agreement with the earlier reports on the same compounds isolated from other sources (Miyazawa et al., 1974; Mahadevappa and Raina, 1978b).

On a total lipid basis, the sterol lipids comprised free sterols, 0.13, esterified sterols, 0.024, sterol glycoside, 0.036, and esterified sterol glycoside, 0.029% weight, respectively.

Table I shows that esterified sterol was more unsaturated than the esterified sterol glycoside (calculated iodine values of 139 and 93, respectively). In both esterified sterol and esterified sterol glycoside, linoleic acid, linolenic acid, and palmitic acid were predominant. The percentage of total unsaturated fatty acids was high in both esterified fractions (69% in esterified sterol and 53.6% in esterified sterol glycoside).

Table II shows the percentages of different sterols present in free sterol, esterified sterols, sterol glycoside, and esterified sterol glycoside fractions. The gas chromatogram showed only three peaks, identified as β-sitosterol, stigmasterol, and campesterol, the last in very small proportions.

Paper chromatograms of the methyl glycosides prepared from sterol glycoside and esterified sterol glycoside indicated that D-glucose was the major sugar in both. Gas chromatographic resolution of the trimethylsilyl ether derivatives of these methyl glycosides yielded only two peaks corresponding to methyl α- and β-glycosides, confirming that glucose is the only component sugar moiety in both sterol glycoside and esterified sterol glycoside.

DISCUSSION

In all the four sterol-containing lipid fractions, β-sitosterol and stigmasterol were the major components accompanied by a small percentage of campesterol. When the esterified sterol fraction is considered, since linoleic, palmitic, and linolenic acids constitute about 42, 25, and 20% respectively, the molecular species present would mainly be β-sitosterol or stigmasterol linoleate and to a lesser extent palmitate and linolenate. All the three molecular species are possible. Glucose is the only sugar

Table II. Sterol Composition of Four Sterol-Containing Lipid Fractions (Percent Weight)

sterols	rel GLC retention time	free sterol fraction	esterified sterol fraction	sterol glycoside fraction	esterified sterol glycoside fraction
β-sitosterol	100	58.6	60.2	55.4	54.0
stigmasterol	84	38.5	37.0	40.0	42.0
campesterol	80	2.9	2.8	4.6	4.0

component in sterol glycosides, and hence the molecular species present in them are likely to be β-sitosterol or stigmasterol glucoside and sterol 6-acylglucoside, where the sterol could be β-sitosterol or stigmasterol and the acyl moiety palmitic, linoleic, and linolenic acid, respectively. On the basis of the earlier reports (Kiribuchi et al., 1966; Miyazawa et al., 1974; Mahadevappa and Raina, 1978b), the structure deduced for sterol glycoside would be β-D-glucopyranosyl-(1→3)-S, where S represents either β-sitosterol or stigmasterol and acyl would imply palmitic, linoleic, or linolenic acid.

The main sterol in plant sources reported earlier (Lepage, 1964; Kiribuchi et al., 1965; Sakata et al., 1973; Ito and Fujino, 1974; Miyazawa et al., 1974; Mahadevappa and Raina, 1978b; Fujino, 1978) is β-sitosterol with lesser proportion of stigmasterol. This is true of this legume also. Campesterol here constitutes about 3% which is in agreement with the earlier findings on pea and soybean (Miyazawa et al., 1974; Kiribuchi et al., 1966). It has earlier been established by using a cell-free particulate fraction of immature soybean seeds that sterol glycoside is biosynthesized from β-sitosterol and UDP-glucose. There is also a possibility that esterified sterol glycoside is formed likewise (Hou et al., 1967; Kates, 1970). A similar biosynthetic pathway for the formation of sterol glycoside and esterified sterol glycoside seems to be operating in this legume also. Stigmasterol could presumably substitute for β-sitosterol in the biosynthetic pathway to yield corresponding molecular species.

ACKNOWLEDGMENT

The authors thank Drs. B. L. Amla, M. R. Raghavendra Rao, and K. T. Achaya for their interest and advice.

LITERATURE CITED

Carter, H. E.; Gaver, R. C. *J. Lipid Res.* **1967**, *8*, 391.
Fujino, Y. *Cereal Chem.* **1978**, *55*, 559.
Hou, C. T.; Umemura, Y.; Nikamura, M.; Funahashi, S. *J. Biochem. (Tokyo)* **1967**, *62*, 389.
Ito, S.; Fujino, Y. *Nippon Nogei Kagaku Kaishi* **1973**, *47*, 229; *Chem. Abstr.* **1974**, *80*, 68380.
Kates, M. *Adv. Lipid Res.* **1970**, *8*, 225.
Kiribuchi, T.; Chea San Chen; Funahashi, T. *Agric. Biol. Chem.* **1965**, *29*, 265.
Kiribuchi, T.; Mizunaga, T.; Funahashi, S. *Agric. Biol. Chem.* **1966**, *30*, 770.
Lepage, M. *J. Lipid Res.* **1964**, *5*, 587.
Mahadevappa, V. G. Ph.D. Thesis, Mysore University, India, 1980.
Mahadevappa, V. G.; Raina, P. L. *J. Agric. Food Chem.* **1978a**, *28*, 1241.
Mahadevappa, V. G.; Raina, P. L. *J. Am. Oil Chem. Soc.* **1978b**, *55*, 648.
Miyazawa, T.; Ito, S.; Fujino, Y. *Cereal Chem.* **1974**, *51*, 623.
Rouser, G.; Kritchevsky, D.; Yamamoto, A. "Lipid Chromatographic Analysis"; Marcel Dekker: New York, 1967; Vol. 1, pp 99–162.
Sakata, S.; Ito, S.; Fujino, Y. *Nippon Nogei Kagaku Kaishi* **1973**, *47*, 125; *Chem. Abstr.* **1973**, *79*, 2773.

Received for review January 9, 1981. Accepted August 10, 1981.

Study of γ-Irradiated Starches Derived from Different Foodstuffs: A Way for Extrapolating Wholesomeness Data

Jacques J. Raffi,* Jean-Pierre L. Agnel, Corinne J. Thiery, Claudine M. Frejaville, and Louis R. Saint-Lèbe

The foodstuff from which the investigated starches (maize, amylomaize, waxy maize, bread wheat, manioc, rice, potato, and haricot bean) derive only has a little influence upon their γ irradiation: the intermediate free radicals as the final radiolysis products are the same; the ratio between the maximal and minimal formation yields is always lower than 5; the different curves drawn (influence of irradiation dose, atmosphere, or water content) have similar shapes.

For reduction of food losses and for improvement of their nutritional quality, many approaches are used. Irradiation of food is among one of the recent ones. The appropriate studies required to ascertain the wholesomeness of irradiated food were discussed by different Joint FAO/IAEA/WHO Expert Committees (JECFI) in 1964, 1969, 1976, and 1980. In view of advances of knowledge, the 1976 Committee suggested (WHO, 1977) that "*Future evaluations of the wholesomeness of individual irradiated foods should take into consideration all relevant data obtained from tests on analogous irradiated foods and on repesentative food constituents*". Moreover, it is envisaged that radiation chemical investigations will eventually provide sufficient data to facilitate greatly the evaluations of irradiated foods. For instance, "*It was considered reasonable to take into account the radiation chemical studies on various starches and the absence of adverse effects in feeding studies with irradiated maize starch*" (WHO, 1977). Our laboratory, like others taking part in the CORC program (coordinated program in the field of radiochemistry of food and food components) of the IFIP (Inernational Food Irradiation Project), agreed that chemiclearance (Basson and Elias, 1978) was a rational approach to ascertain the wholesomeness of irradiated food. This work is designed to determine if there are variations in the amounts of radiolysis products from different starches. Our proposed method was not to undertake a chemical study as systematic as in the case of maize starch (Berger et al., 1977; Raffi et al., 1978); however, such a study would have been tedious and even unprofitable, our point being only to verify that the differences noticed after irradiation of these starches derive from various physical properties (degree of polymerization crystallinity, ...).

In addition to chemical studies, we also used electron spin resonance experiments in order to observe radioinduced radicals directly and to follow the influence of the

Groupe de Radioconservation des Denrées, Service de Radioagronomie, C.E.N. Cadarache, B.P. No. 1, 13115 Saint-Paul-Lez-Durance, France.

0021-8561/81/1429-1227$01.25/0